Technical Guide

for the Elaboration of Monographs

European Pharmacopoeia

European Directorate for the Quality of Medicines

4th Edition - 2005

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TECHNICAL GUIDE FOR THE ELABORATION OF MONOGRAPHS
4th Edition – 2005

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TECHNICAL GUIDE FOR THE ELABORATION OF MONOGRAPHS

1 INTRODUCTION

1.1 PURPOSE OF THE GUIDE

This document is a guidance for the authors of monographs and also a means of communicating to the users of the European Pharmacopoeia, especially industry, licensing authorities and official medicines control laboratories, the principles for the elaboration of monographs. Since the principles applied and guidance given for the elaboration of monographs should be the same as those applied by licensing authorities the Technical Guide may also serve as a guideline in the elaboration of specifications intended for inclusion in licensing applications.

It is necessary to bear in mind that a monograph will be mandatory standard and must be applicable in licensing procedures in all Member States of the Convention on the Elaboration of a European Pharmacopoeia. The procedures for the tests and assays in the individual monographs must therefore have been validated according to the current practice at the time of their elaboration.

1.2 TEST PROCEDURES

The methods chosen for the identification tests, purity tests and assay(s) constituting the bulk of a pharmacopoeial monograph are preferably those already described and utilised in the European Pharmacopoeia. In this context the author of a monograph is referred, not only, to the General Methods of the Ph. Eur. but also to published monographs on similar materials. The above considerations aim at ensuring a reasonable degree of harmonisation within the Pharmacopoeia and they only apply in cases where the methods are found to be adequate for the specific purposes. However, due attention is also to be paid to the development of new methods that offer significant improvements in terms of sensitivity, precision, accuracy or discriminating power (selectivity).

Methods included in monographs must be validated as described in the section on analytical validation and other relevant specific sections of this guide. Validation reports are provided to EDQM but are not published or otherwise provided to users.

The test procedures included in a monograph should be verified in two or more laboratories and the laboratory reports on this verification should be provided to EDQM to ensure future traceability.

The instructions describing any method of analysis cover all factors that can influence the results and that are deemed essential to enable an experienced analyst working according to acknowledged laboratory practices, yet without necessarily having any prior knowledge of the investigation in question, to perform the analysis. Variations in the description of similar methods are to be avoided.

If an analytical procedure is, or may be, expected to be used generally or if it requires a lengthy description and is used more than once, it may be proposed for inclusion in the general chapters of the Pharmacopoeia, to be referred to in the individual monographs. The methods are prescribed on the scale conventionally applied in the Pharmacopoeia except in cases where for reasons of availability of the material to be analysed, or because of its toxicity or its cost, work on a small scale would be advantageous.

1.3 EQUIPMENT

If the equipment utilised for a method of analysis is not generally available in the States party to the European Pharmacopoeia Convention, it must be possible to have it constructed according to its description in the Pharmacopoeia.

1.4 QUANTITIES

In prescribing the quantities, i.e. masses and volumes, of substances, reagents, and solvents to be taken for identifications, tests and assays, it is the practice of the Pharmacopoeia to indicate in detail the precision with which they are to be measured, see the General Notices. It is therefore necessary to take this aspect into consideration when drafting Pharmacopoeial texts.

As guidance to minimise errors in the preparation of analytical solutions Table 1, giving estimations of the relative uncertainty, is to be consulted.

In order to avoid either the use of extremely low amounts or an unnecessarily large expenditure of solvents, a dilution series will often have to be prescribed for the preparation of dilute solutions used particularly for spectrophotometric measurement. In this context not all combinations of (usually two or three) dilution steps will contribute equally to the random error of the dilution procedure. If critical for the purpose, the optimal dilution is prescribed in consideration of the relative errors (capacity tolerance divided by nominal volume) associated with the various sizes of volumetric pipettes and volumetric flasks commonly used for these operations (taking the usual formula: square root of the sum of the squares of individual relative errors, to estimate the relative dilution error).

Tables giving the optimal number and nature of dilution steps needed to achieve a given dilution ratio, based upon given specifications for the capacity tolerances of volumetric glassware, are available in the literature. For guidance see Table 2 (it is to be noted that these factors do not include reading errors).

Table 1 — Relative Uncertainties in the Preparation of Analytical Solutions

Concentration to be prepared	Preparation of solution	Percentage Relative Uncertainty		
		Mass	Volume	Total
10 g/1000 ml	10g/1000ml	< 0.01	0.05	0.05
	1g/100ml	0.02	0.12	0.12
	0.5g/50ml	0.04	0.17	0.17
	0.25g/25ml	0.08	0.23	0.24
	0.1g/10ml	0.02	0.50	0.54
1g/1000 ml	1g/1000ml	0.02	0.05	0.05
	0.5g/500ml	0.04	0.07	0.08
	0.25g/25ml	0.08	0.23	0.24
	100mg/100ml	0.2	0.12	0.23
	50mg/50ml	0.4	0.17	0.43
	10mg/10ml	2.0	0.50	2.06
0.1 g/1000 ml	100mg/1000ml	0.2	0.05	0.21
	50mg/500ml	0.4	0.07	0.41
	25mg/250ml	0.8	0.08	0.80
	10mg/100ml	2.0	0.12	2.0
	5mg/50ml	4.0	0.17	4.0
	1mg/10ml	20.0	0.50	20.0
0.01 g/1000 ml	10mg/1000ml	2.0	0.05	2.0
	5mg/500ml	4.0	0.07	4.0
	1mg/100ml	20.0	0.12	20.0

An uncertainty of 0.2 mg for the weighing procedure has been assumed for the calculations of the percentage relative uncertainties.

Table 2 — Relative errors for dilution with analytical glassware (pipettes P/flasks F)

Concentration ratio	No. of steps	Step 1		Step 2		Relative error
		P	F	P	F	
1/2	1	25	50			0.16
1/2.5	1	20	50			0.18
1/5	1	20	100			0.17
1/10	1	25	250			0.13
1/12.5	1	20	250			0.16
1/30	1	15	500			0.20
1/50	1	20	1000			0.15
1/100	1	25	250	25	250	0.18
1/125	2	20	250	25	250	0.20
1/160	2	25	1000	25	100	0.19
1/200	2	25	500	25	100	0.18
1/250	2	20	250	25	500	0.20
1/400	2	25	250	25	1000	0.18
1/500	2	20	500	25	500	0.20
1/1000	2	20	1000	25	500	0.20

Adapted from R B Lam and T L Isenhour, Minimizing relative error in preparation of standard solutions by judicious choice of volumetric glassware, *Analytical Chemistry*, 1980, **53**, 1158-1161.

1.5 REAGENTS

When the quality of a reagent substance in one or more respects is critical for its intended use it must be carefully defined, when necessary by prescribing appropriate tests to demonstrate its suitability. Normally analytical grade reagents are employed in which case it is sufficient to give the name of the reagent, the CAS number and its formula.

Whenever possible the reagent substances, reagent solutions, volumetric solutions and standard solutions for limit tests already described the reagents chapters of the European Pharmacopoeia are to be employed. Simple solutions of reagent substances or solutions that are prepared for use on a single occasion are to be described in the monograph itself.

The use of reagents, that are acknowledged to be extremely toxic or otherwise hazardous (e.g. carcinogenic), is to be avoided, especially in circumstances where their dangerous properties are difficult to control, e.g. when handled as fine powders or in spray reagents. The use of a number of substances which are prohibited or restricted in one or more of the States party to the European Pharmacopoeia Convention, are also to be avoided.

1.6 COMMERCIAL NAMES

Commercial names should be given as footnotes in draft monographs systematically for chromatography columns/plates and in other cases wherever it will be useful for analysts (test kits, reagents that are available from a single supplier etc.). Commercial names are not included in the text published in the Pharmacopoeia but are transferred to the EDQM web site Knowledge database after adoption of the monograph.

1.7 REFERENCE STANDARDS

The policy and procedures regarding reference standards are described in general chapter *5.12. Reference standards* (at present in draft form). Procurement, establishment, storage and monitoring of reference standards are the responsibility of EDQM. Many reference standards, notably those for control of impurities, are available only in limited quantities. Before publication of a monograph in Pharmeuropa, the required quantities of reference standards should be supplied to EDQM, who will also advise on the best strategy for optimising the use of substances that are available in limited quantities (for example, preparation of a spiked substance rather than supply of the single substance). The aim of EDQM is to present the reference standard for adoption at the same time as the monograph or, failing that, by the time of publication at the very latest.

From the 5th Edition onwards, a change was made to the policy for establishment of an IR reference spectrum, which was previously the option of choice where the only use for a reference standard was IR identification. Preference is now given to chemical reference substances over reference spectra, except in special cases, for example where provision of a reference substances entails practical difficulties.

Many reference standards are available in limited quantities, notably impurities, and the amount prescribed for preparation of solutions must be kept to a minimum.

2 MONOGRAPH ON A SUBSTANCE FOR PHARMACEUTICAL USE

Monographs are based on the specifications for substances used in medicinal products approved in Member States. When a monograph is added to the work programme enquiries are made by EDQM to identify manufacturers of such substances and all data received is taken into account for preparation of the monograph. Interested parties should be invited to participate in the elaboration of the monograph before publication in Pharmeuropa, since the 3-month public period will often be too short for all interested parties to check the draft monograph.

Prior to the preparation of any monograph it is essential to gather as much information as possible on the substance in question.

In particular it is necessary to ascertain:

— whether the substance is of natural, synthetic or semi-synthetic origin;

— whether the substance is a mixture or a single entity;

— the method(s) of preparation in detail;

— whether there are different crystalline forms, since the properties of the substance may vary in accordance with this parameter;

— whether both an enantiomer as well as the racemate or other mixtures of enantiomers are available;

— whether different hydrates are available;

— whether different entities (acid, base, salt, etc) are available.

The Pharmacopoeia and other relevant documents on the state of work must be consulted to see if monographs on similar substances exist or are being elaborated. If monographs or drafts on similar substances already exist, it is important to ensure that the monograph to be elaborated follows the same approach unless there are good reasons to deviate, e.g. developments in analytical techniques.

Substances that are to be described in a monograph may be members of a group of very similar substances (family). This holds true especially for excipients such as macrogols. A master monograph is to be drafted clearly stating the attributes common to all members of the family and which can be used to identify single members of the family (family monograph).

All active substances and excipients described in the European Pharmacopoeia are subject to the provisions of the general monograph *Substances for Pharmaceutical Use (2034)*.

*Title.*The International Nonproprietary Name (INN) established by the World Health Organisation should be used wherever it is available; it is supplemented as appropriate by the name of the anion or cation and by “hydrate”, “dihydrate”, “hydrated” (for ill-defined degrees of hydration) or “anhydrous” (where a hydrated form is also known to exist). Formerly, the degree of hydration was not indicated in titles unless two forms were known to be available; existing titles of this type are not changed on revision unless it is known that two forms are available or if there is a public health imperative (for example, high water content that could lead to errors in formulation). Anions and cations are indicated as “mono-“, “di-“, tri-“, as appropriate.

Where a substance is used in approved medicinal products for veterinary use only in Member States, “for veterinary use” is included in the title.

2.1 DEFINITION

The chemical structure must be ascertained with the greatest possible precision in order to establish the exact:

— graphic formula;

— empirical formula and relative molecular mass;

— chemical name. This implies investigating in particular:

— the possible existence of isomers so as to be able to specify which isomer is used or, otherwise, to state that the product is a mixture of isomers;

— In the case of an optical isomer, it is insufficient to take into account only the direction of the optical rotation. The absolute configuration is given by the R/S system at the asymmetrical centre(s) or any other appropriate system (eg. for carbohydrates and amino acids);

— ascertaining the state of hydration or solvation so as to distinguish clearly between the well-defined hydrates and solvates and the products that contain variable quantities of solvent(s). As regards the former, water or solvent content ranges are specified but for the latter only a maximum content is given. When a substance exists both in a water-free or solvent-free form and in the form of (a) hydrate(s) or (a) solvate(s) with different water or solvent contents, and if all these forms are used, they are normally treated as individual substances requiring separate monographs.

Some chemical substances, particularly those obtained from raw materials of natural origin and substances produced by fermentation may not be easily separated from certain related substances (for instance, quinine salts). These may be treated as:

— a chemical product when obtained in a very pure state and when they can be assayed by a physico-chemical method;

— a substance accompanied by a certain proportion of related substances, giving an exact definition of the main component only (e.g., neomycin);

— a mixture of several components, sometimes difficult to define, where an overall description may suffice (e.g., nystatin).

Where applicable the origin of the substance must be specified (name and strain of the organism from which the substance is derived). Where applicable, the monograph indicates that the substance is semisynthetic and derived from a fermentation product [to clarify application of the monograph on *Substances for Pharmaceutical Use (2034)*].

2.1.1 Combinations

In therapeutics, more or less well-defined chemical combinations (for instance, theophylline - ethylenediamine) or even mixtures are sometimes used. In such cases, it is necessary to specify precisely each component of the combination or mixture, with its chemical structure and the proportion in which it is present.

2.1.2 Content

The substance described by a monograph is never a wholly pure substance, but contains a limited proportion of impurities. The content is therefore an important part of the definition. Assay limits are specified between which the content must fall. The assay limits must take account of the precision of the method as well as the acceptable purity of the substance. Assay limits are normally expressed with reference to the dried or anhydrous substance; correction for residual solvent is understood [see *Substances for Pharmaceutical Use (2034)*].

For a non-specific assay (for example, titrimetry) the assay limits are usually 99.0 - 101.0 per cent (unless otherwise justified). For a specific assay using a separation technique (for example, liquid or gas chromatography), the upper assay limit is normally 102.0 per cent; the lower assay limit will take any necessary account of the impurities present and may therefore be lower than 98.0 per cent.

In setting these limits for the active ingredient content, account is taken of:

— the method of preparation, which determines the degree of purity which may be reasonably required;

— the reproducibility and accuracy of the analytical method;

— where a separation technique is employed both for the test for related substances and the assay, content limits are set taking into account the maximum permitted amount of impurities and the analytical error;

— the evaluation of the tolerable degree of deterioration during storage;

— a sufficient number of experimental results obtained on several batches (at least 3), if possible, of different origins and ages.

When the substance to be examined contains only impurities that do not interfere with the assay, or when it contains only a very low proportion of impurities interfering with the assay, the results of the assay can be used directly. It will then be stated that: the substance contains not less than x per cent and not more than the equivalent of y per cent (at least 100.5 per cent, but often a little more) of... (chemical definition of the pure product). The content of the substance is usually expressed with reference to the anhydrous or dried substance. In certain cases, it is necessary to express the content on a solvent-free basis or a solvent-free and anhydrous basis. The general monograph on *Substances for Pharmaceutical Use (2034)* has a provision for calculation of content with reference to the solvent-free substance, which covers cases where the test for residual solvent is not included in a specific monograph.

When the substance to be examined contains a relatively large proportion (a few per cent) of impurities, which are determined at the same time as the active ingredient, an appropriate wording is to be used (for instance, in the case of quinine salts ... x per cent of total alkaloid salts, expressed as quinine salts...).

Exceptionally reference is made to only a part of the molecule or to an element (for example, assay of magnesium oxide in light magnesium carbonate or assay of magnesium in magnesium stearate).

In the case of antibiotics determined by microbiological assays, the active ingredient content is expressed in International Units, where these exist, and a minimum value only is given.

See also section 2.5 Assay.

2.2 CHARACTERS

As defined in the General Notices, statements under the heading CHARACTERS are not to be interpreted in a strict sense and are not regarded as analytical requirements.

The principal items that may be referred to under this heading are the following.

2.2.1 Appearance

This description will normally embrace colour and physical form. The term "white" is not used without qualification since, if viewed against a standard white material, very few pharmaceutical materials will appear truly white. It is, of course, not intended that such a comparison be made but experience shows that certain users of the Pharmacopoeia may insist on doing so as part of a purchasing contract. The term "white or almost white" is used instead. Where positive colours are to be described this is done in terms of primary colours or combinations of primary colours.

Colour: The following descriptive terms are used:

black	green	red
blue	grey	violet
brown	orange	
colourless	pink	yellow
white/almost white		

Compound terms may be used:

English	French
greenish-blue	bleu-vert
bluish-green	vert-bleu
violet-red	rouge-violet
reddish-violet	violet-rouge
brownish-red	rouge-brun
reddish-brown	brun-rouge

In English, the dominant is placed second, whereas in French, it is placed first. Expressions such as lemon-yellow, buff, salmon-pink are to be avoided; standard dictionaries give equivalents for such terms as spectral colours with suitable qualifiers (for example, buff is described as 'dull yellow'). The following adjectives are also used; light, slight, fluorescent, intense, pale, dull, deep, dark.

It is to be noted that the allowed colours and colour combinations also apply to the description of the colour changes of indicators when used in acid/alkalinity tests or in titrimetric assay procedures.

2.2.2 Taste

The taste is not to be taken into consideration.

2.2.3 Odour

In general, no reference is made to odour. In particular no reference to odour is made for those materials that would constitute a hazard if inhaled. Mention of odour in other cases must be justified.

2.2.4 Solubility

A method recommended for the estimation of solubility is given in general chapter *5.11 Characters section in monographs*. All solubilities are quoted in the general terms defined in the General Notices. Solvents quoted are normally confined to water, alcohol and a lipophilic solvent. Solubilities in chloroform and ether are not mentioned. In special cases the solubility of different samples of a material may vary rather considerably even though their composition is still within the limits set by the monograph. The solubilities in the solvents thereby affected are then given to cover more than one solubility class, e.g. "sparingly soluble to soluble in etc.". The solubilities or miscibilities in other solvents with which the material is often combined in practice such as fatty oils etc. may also be mentioned. In some cases it may be useful to specify solubility in alkalis or acids and, particularly in cases of materials that are very insoluble in the above mentioned solvents, a special solvent may be indicated, eg dimethylformamide or dimethyl sulphoxide. It is not necessary to specify the solubility in every solvent that is used in performing the tests of the monograph itself.

2.2.5 Stability Factors

Evidence of instability due to exposure to air, light and for moisture is to be given e.g. physostigmine sulphate turns red when exposed to air and light. Any such statement under CHARACTERS is given separately from the description of a pharmacopoeial material.

2.2.6 Hygroscopicity

A pragmatic method recommended for the determination of the tendency of a substance to take up atmospheric water (rather than a true determination of hygroscopicity)is given in general chapter *5.11 Characters section in monographs*. Some substances are hygroscopic or deliquescent which results in difficulty for the analyst during weighing procedures. In such cases, this is indicated using the terminology defined under *5.11*. for information of the analyst as an alert for precautions to be taken in handling the substance.

Where a substance is described under Characters as hygroscopic or deliquescent, storage in an airtight is indicated.

2.2.7 Solid-state properties

Solid-state properties include crystallinity, polymorphism, density of solids, particle size of solids and specific surface area of solids. Solid-state properties, particularly polymorphism and pseudopolymorphism, may have an effect on the bioavailability of the substance and for the production of the medicinal product. General chapter *5.9 Polymorphism* should be consulted.

A method recommended for the determination of crystallinity is given in general chapter *5.11 Characters section in monographs*.

Solid-state properties of excipients that are relevant for functionality may be dealt with in the section Functionality-related Characteristics (see below).

A statement in the pharmacopoeia of the occurrence of polymorphism is intended to alert users to the need to evaluate this phenomenon during the development of a dosage form.

When polymorphism is known to exist in the substance, then this information is given as a separate statement ("it shows polymorphism").

Two cases are to be distinguished when polymorphism is known to exist :

- Usually, the monograph does not exclude any of the possible crystalline forms.
- Exceptionally, if the substance is only used in solid dosage forms and one form has been shown to be preferred from the point of view of bioavailability or to have a better safety/efficacy profile then the monograph may be limited to that form. The techniques required to identify the form are included in the « Identification » section.

2.2.8 Other Characteristics

Other physical characteristics that may be useful as information but not sufficiently precise to be defined under the headings IDENTIFICATION or TESTS may be stated under the heading CHARACTERS. This will usually apply to a melting point that is insufficiently precise to allow a range to be quoted. (If a range can be quoted the melting point may be included under IDENTIFICATION). When decomposition may occur, this must be stated. Other general characteristics that may be of relevance for quotation under the heading CHARACTERS include an indication of direction of optical rotation in a particular solvent or, in the case of radio-active materials, a note of the half-life of the radionuclide defined and of the type of radiation that it emits.

2.2.9 Behaviour in Solution

In cases where it is known that rapid degradation may occur in solution, this information is given as a warning statement.

2.3 IDENTIFICATION

2.3.1 General

The purpose of the IDENTIFICATION section of a monograph is to provide confirmation of the identity of the substance in question. Identification according to the Pharmacopoeia is thus generally of a much more limited scope than the structural elucidation of an unknown substance or the determination of the composition of an unknown mixture. The task of identifying a material is not to be confused with the assessment of its purity or the determination of its strength, although ultimately all three aspects are complementary.

It follows from the above that the physical and/or chemical tests and reactions when taken together, that enter into the IDENTIFICATION section ensure, as far as possible, specificity. The specificity of the identification should be such that active substances and excipients exhibiting similar structures are distinguished. They are not to be too sensitive, i.e. false reactions caused by the presence of tolerated impurities are to be avoided and they do not require more experimental effort than necessary for differentiating the substance in question from other pharmaceutical substances available in commerce. In consideration of experimental effort, the time needed to perform a test is also taken into account.

Normally, a single set of tests for identification is given. Where justified, in order to give users of the Pharmacopoeia a choice between methods requiring complex instrumentation

and other methods, two sets of identification tests may be included. This is usually the case when the substance is used in hospital and/or community pharmacies. Monographs then have subdivisions entitled 'First identification' and 'Second identification'. The test or tests that constitute the 'Second identification' may be used instead of the test or tests of the 'First identification' provided it can be demonstrated that the substance or medicinal product is fully traceable to a batch certified to comply with all the requirements of the monograph.

Some of the purity tests in a monograph may also be suited for identification purposes, possibly in a modified form. A system of cross-reference to the TESTS section can be exploited. This is particularly relevant in cases where distinction between closely related materials depends on properties that are also parameters in purity or composition control e.g. water content of different hydrates, optical rotation of different isomers, the viscosity of chain-length homologues of a polymer. The IDENTIFICATION section is self-sufficient in the monograph even if this includes cross-reference to other sections.

The monograph of a substance must not be treated in isolation. When an identification series is being investigated it is desirable that other similar substances, whether or not they are the subject of monographs of the pharmacopoeia, are examined at the same time to ensure that a particular combination of tests within a series will successfully distinguish one similar substance from another. Such a validation of the IDENTIFICATION section must always be carried out.

In the case of a family monograph, identification of the type of substances may be supplemented by non-specific but discriminating tests to identify individual members of the family.

Examples are given below of some methods of identification and they are followed by detailed guidelines concerning some of them.

2.3.1.1 methods requiring complex instrumentation

- Spectrophotometric analysis, such as recording of infrared or nuclear magnetic resonance spectra.
- Chromatographic examination by means of gas chromatography or liquid chromatography.

2.3.1.2 other methods

- Determination of physical constants such as melting point, freezing point, boiling point, specific optical rotation, angle of rotation, ultraviolet spectrum, specific absorbance, relative density, refractive index and viscosity.
- Chemical reactions such as colour or precipitation reactions (including formation of derivatives or degradation products, which may subsequently be subjected to physical examination) and determination of chemical values (saponification, ester, hydroxyl and iodine values).
- Chromatographic examination by thin-layer chromatography.

2.3.2 Infrared Absorption Spectrophotometry

This is generally considered to be a satisfactory single method for verification of the identity of non-ionised organic substances other than salts of organic acids or bases. This method always necessitates the use of a reference substance or a reference spectrum. Reference substances are now preferred to reference spectra. The latter are used where there are practical difficulties with providing a reference substance.

Organic salts of organic substances and some inorganic salts of organic substances (e.g. phosphates and sulphates) can readily be distinguished from each other. In the case of sulphates, however, it is necessary to extend the usual range of recording from 4000 cm^{-1} - 600 cm^{-1} to 4000 cm^{-1} - 400 cm^{-1}.

The method of sample preparation (disk, halide salt plate, mull, etc) is not specified unless this has been found to be necessary during development of the monograph for obtaining a satisfactory spectrum.

In certain cases, there is a need to supplement the infrared spectrum with other tests where the spectrum alone is insufficient for confirmation of identity as follows.

2.3.2.1 salts of organic acids or bases

For several ions or groups that form part of an organic substance, more than one identification test is described amongst the general methods. However, it is usually only necessary to utilise one of them.

2.3.2.2 chemically related substances

When substances closely related to the substance under examination exhibit variations in the spectra which are considered insufficient for unambiguous identification. In such cases the infrared spectra are accompanied by another simple test e.g. melting point or thin-layer chromatography with the use of a reference substance.

2.3.2.3 polymorphism

The general chapter allows for recrystallisation before recording of the spectrum. Where a monograph mentions polymorphism then a method for recrystallisation is described unless it is the intention to limit the scope of the monograph to the crystalline form represented by the chemical reference substance In the latter case the monograph indicates that the spectrum is recorded “without recrystallisation”.

Exceptionally when the monograph describes a specific crystalline form or forms and when the IR spectrum is not characteristic, an additional test is introduced.

2.3.2.4 optical isomers

To identify a particular isomer or a racemate the test for specific rotation or angle of rotation is added.

2.3.3 Ultraviolet and Visible Absorption Spectrophotometry

This method is usually non-specific for identification purposes, unlike infrared spectrophotometry, unless the absorption curve exhibits several maxima and minima,

unusually strong or weak regions of absorption etc. Reference substances are generally not used. The UV spectrum of a substance can, therefore, seldom stand on its own as an identification criterion.

The concentration of the solution to be examined is such that the absorbance preferably lies between 0.5 and 1.5, measured in a 1 cm cell.

The range of wavelengths to be explored must be stated; generally it does not extend towards the region where end-absorption and solvent interference may be expected. The wavelengths of sharp maxima and minima are indicated by a single number, signifying ±2 nm, whilst for broader bands a range is given. When it is considered necessary to mention the wavelength of shoulders etc, the term "about" may be used.

Specific absorbances are also given as a range (usually ±5 %) in order to cover variations in content of absorbing substance and experimental error. It is to be noted that the instrument tolerance for absorbance is ± 0.01, which means that the percentage deviation due to this source of variability will depend upon the absolute levels of absorbance. Furthermore, the content of absorbing substance will vary with the permitted content of water (or other solvents); when the latter does not exceed 1 per cent or is within well-confined limits it will usually be adequate to calculate the specific absorbance for the substance "as is" and to set the limits accordingly. When more than a single maximum is present in the spectrum the ratio(s) between their absorbances can be substituted for the individual specific absorbances, providing the ratio is less than or equal to 5, thus avoiding having to correct the absorbances for the solvent content of the substance.

Care must be taken in the choice of solvents and solvent purity prescribed for ultra-violet spectrophotometry in order to avoid the presence of impurities, which may influence the absorbance of the substances to be examined.

In certain cases of identification by means of absorption spectra in the UV-VIS range, the resolution of the instrument can be expected to constitute a critical factor in observing the required spectral features (e.g. benzenoid type spectra showing fine structure). In certain cases the minimum resolution required is indicated in the monograph. In order to determine this figure the slit width setting is deliberately varied to the point where the spectrum obtained is just adequate for the intended purpose. The resolution corresponding to this setting is then experimentally defined on the basis of an absorbance ratio for a 0.02 per cent V/V solution of toluene R in hexane R as prescribed in the general method (*2.2.25*). The minimum ratio is indicated in the monograph with two significant figures.

Table 3 indicates, for information purposes, the approximate relationships to be expected between the spectral slit width and the absorbance ratio.

Table 3 — Resolution of Spectrophotometers according to the Slit Width

Slit Width (nm)	Ratio A_{max} 269 nm/A_{max} 266 nm
0.25	2.3
0.5	2.2
1.0	2.0
2.0	1.4
3.0	1.1
4.0	1.0

2.3.4 Melting point, Freezing point and Boiling point

These physical constants are of value in identification only if they are well defined and their determination is not accompanied by destruction to a degree that renders them extremely dependent on the actual mode of operation. The possible existence of polymorphism must also be taken into account; differences in the melting point must be indicated even when given under CHARACTERS. In exceptional cases, when the distinction of a specific form is necessary, determination of the melting point can aid in excluding the unwanted form(s).

However, it should be kept in mind that an apparent melting point may be observed: a solid-solid polymorphic transition may take place during testing and the melting point of the resultant form is measured

Neither the melting point alone nor the addition of a chemical reaction is sufficient to confirm identity of a substance. However, the addition of another identification test such as TLC, will often suffice. The melting point determined by the usual capillary method is defined in the Pharmacopoeia as the last particle melting point. It must not be confused with the melting interval even though both are given as a range.

2.3.5 Specific Optical Rotation

When an enantiomer is described in a monograph, a test for optical rotation is given in the IDENTIFICATION section or a cross-reference is made to the test for enantiomeric purity in the TESTS section. When both the racemate (or the racemic mixture) and the enantiomer are available then, in the monograph of the racemate, an angle of rotation will be given in the TESTS section and will be referred to in the IDENTIFICATION section. When only the racemate is available the angle of rotation will be given in the TESTS section, provided the specific optical rotation of the chiral form is known and is of sufficient magnitude to provide a meaningful test for racemic character.

2.3.6 Thin-layer Chromatography

This identification method requires the use of reference substances. Selectivity may be improved by combining thin-layer chromatography with chemical reactions in situ i.e. by

employing appropriate spray reagents. In the latter case, the same or a similar reaction is not to be repeated on a test-tube scale.

It is very important to assure the separation of a critical pair in a related substances test, but such a separation plays a minor role in an identification test. The separation of a critical pair in the individual IDENTIFICATION tests is no longer required but the separation of a critical pair in the TEST section is maintained. However, during development and validation separation of the substance from similar substances must be demonstrated.

A chromatographic separation test is described in the Reagents Section for verifying performance of the plate type concerned. The test is intended to be a quality control procedure, carried out from time to time by the TLC plate user. It is clear that such a general procedure is not representative for every thin-layer separation problem and that the description of a separation criterion might still be necessary to ensure the identification of the substance. In these exceptional cases a separation criterion is described in the IDENTIFICATION section.

A TLC system applied to purity testing in a monograph is preferred for identification when suitable. For the latter purpose the concentration of the solution to be examined and the corresponding reference solution is generally reduced so that 5 to 20 µg of each is deposited on the plate or sheet. It may also be necessary to change from a general to a more discriminating detection system.

Further technical guidelines on these chromatographic methods are to be found in the sub-section concerning related substances.

2.3.7 Gas Chromatography and Liquid Chromatography

The basic principles mentioned under thin-layer identification apply *mutatis mutandis*. Gas and liquid chromatography are rarely used for identification and where they are applied it is by reference to a test or assay that applies the method elsewhere in the monograph. These methods are used only if there is no suitable alternative and are not used as the only identification test. Further technical guidelines on gas and liquid chromatography are to be found in the sub-section concerning related substances.

2.3.8 Chemical Reactions

Several commonly applied identification reactions of a chemical nature are included amongst the general methods of the Pharmacopoeia and these are to be utilised, whenever appropriate. Where several reactions for an ion or group are given in chapter 2.3.1, it is normally necessary to prescribe only one in the monograph. Attention is drawn to the necessity to specify the amount of material, or solution of it, to be taken for the identification test in question. The same holds true for tests that have to be described in full in the monograph.

Identification criteria that call for the recognition of an odour or a taste are to be avoided.

Each chemical reaction is to be chosen to demonstrate the presence of a different part of the molecule to be identified.

To differentiate substances within a group (family) which differ by:

- the extent of condensation;

- the length of the hydrocarbon chain (eg. fatty acids),

it is necessary to cross-reference to the appropriate purity test(s) where values are determined (e.g. iodine value, saponification value etc.).

2.4 TESTS

2.4.1 General

The TESTS section is principally directed at limiting impurities in chemical substances. General chapter *5.10 Control of impurities in substances for pharmaceutical use* gives details of the policy to be applied.

While it is an essential function of the monograph to ensure adequate purity in the interests of public health, it is not the aim of the Pharmacopoeia to impose excessive requirements that restrict unnecessarily the ability of manufacturers to produce compliant products.

In the interests of transparency, information is included wherever possible on: the impurities controlled by a test; the approximate equivalent (percentage, ppm, etc) of the prescribed limit in terms of the defined impurities or class of impurities. The information on the limit imposed may be a nominal content inferred from the conditions of the test or it may be based on data from recovery experiments.

Certain tests may apply to special grades (parenteral, dialysis solutions, etc) or a test may have a special limit for a particular use: the particular application of a test/limit is indicated within the test.

2.4.2 Titles

Wherever possible, the title includes the impurity or class of impurities limited by the test (e.g. Oxalic acid, Potassium, Copper, Chlorides etc.). Non-specific limit tests carry a more general title appropriately chosen from the standard terminology of the Pharmacopoeia (e.g. Appearance of solution, pH, Acidity or alkalinity, Heavy metals etc.) or a similar designation. Titles that merely refer to the methodology employed in the test (e.g. Absorbance, Specific optical rotation) are to be avoided wherever possible.

2.4.3 Solution S

A solution of the substance to be examined, designated “Solution S”, is prepared whenever this can be used to perform more than one test (and/or identification).

If necessary, several solutions S, (designated S1, S2,...) may be prepared in various ways, each being used for at least two tests.

For insoluble substances, solution S may be prepared by an extraction process.

The solvent used depends on the solubility of the substance to be examined and that of its potential impurities. It may be:

- water (usually);

- carbon dioxide-free water in cases where the presence of carbon dioxide can appreciably influence the outcome of a test e.g. for pH or Acidity or alkalinity (see relevant section),
- distilled water if solution S is used in the tests for barium, calcium and sulphates,
- carbon dioxide-free water prepared from distilled water when both the two aforementioned considerations apply,

- a dilute acid or an alkaline solution;
- more rarely, other solvents (alcohols, tetrahydrofuran ...) that give solutions with a narrower field of application than aqueous solutions.

The solvent used and the concentration chosen depend on the solubility of the substance to be examined and the purpose for which the test is intended. The solvent must make it possible to carry out the specified tests, either directly, or after suitable dilutions explicitly specified in each test. Generally the concentration is around 20 to 50 g/l but may be lower (e.g. 10 g/l) or higher (100 g/l and, exceptionally, more). The quantity of solution S prepared must be sufficient to carry out each of the tests for which it has been prepared. If solution S is to be filtered, account must be taken of the loss on filtering and when the insoluble portion thus separated is to be used for another test, this is clearly indicated.

If several tests can be carried out on the same portion of solution S, this is only done for substances where there are good reasons to economise (expensive products or products whose use is subject to restrictions) and this is then clearly indicated in the monograph.

Depending on the particular tests, the concentration of solution S is defined with varying precision:

- for “Appearance of the solution”, “pH” and some “Identifications”, a precision of 5 to 10 per cent is sufficient;
- for most limit tests a precision of about 2 per cent is appropriate;
- for some cases such as the determination of the specific rotation, the specific absorbance, various chemical values and, more generally, tests where the result is obtained by calculation, a greater precision is needed.

The precision with which the concentration of solution S is defined is that required by the most exacting test for which it is intended.

The description of the preparation of solution S thus specifies:

- the quantity of substance to be examined with the required precision (see General Notices);
- the volume, to one decimal place (10.0 ml, 25.0 ml...) when the concentration must be known to within less than 1 per cent, without a decimal (10 ml, 25 ml...) when a lower precision is adequate.

2.4.4 Appearance of Solution

This test makes it possible to ascertain the general purity of a substance by the detection of impurities insoluble in the solvent selected, or of coloured impurities.

The "Appearance of solution" test is practically always prescribed for substances intended for preparations for parenteral use. Apart from this it is to be applied only if it yields useful information concerning the general purity of the substance.

It can comprise both tests or one only, namely:

- clarity and degree of opalescence of liquids (2.2.1);
- degree of coloration of liquids (2.2.2).

The two tests are practically always carried out on identical solutions, usually solution S, but they may be performed on different solutions.

The solvent employed is usually water but other solvents may be preferred depending on the solubility of the substance to be examined.

When an organic solvent is used to prepare Solution S, it may be necessary to ensure that the solvent also complies with the test, especially where there is a very stringent requirement.

The more concentrated the solution the stricter the test. For very pure substances or those used in high doses, the concentration chosen is 50 to 100 g/l, whereas for less pure substances or substances administered in small doses the concentration is 10 to 20 g/l.

2.4.4.1 clarity and degree of opalescence

This test is mainly performed on colourless substances or those which give only slightly coloured solutions in order to permit valid comparison with reference suspensions.

The quantity of solution required depends on the diameter of the comparison tubes used; it varies from 7 ml to 20 ml for tubes with a diameter of 15 mm to 25 mm prescribed in the general methods. It is, therefore, necessary to take the latter volume into account.

Most often the solution examined must be "clear" (in the Pharmacopoeial sense of the term). However, in certain cases for substances that are not intended to be used in solution, a more marked opalescence may sometimes be permitted.

2.4.4.2 degree of coloration

The test applies to essentially colourless substances that contain, or may degrade to form, coloured impurities that can be controlled by limiting the colour of solution of the substance. Two methods are described in general method (2.2.2) of the Pharmacopoeia:

- Method I only requires 2 ml of solution but it is seldom prescribed except for substances which give highly coloured solutions;
- Method II, which is more discriminating and therefore more frequently used, requires the larger volume of solution employed for the clarity test.

The results given by these two methods do not necessarily coincide so the one to be used is specified in the monograph.

The solution is described as colourless when it is less coloured than reference solution B9. When the solution is slightly coloured, the appropriate reference solution (2.2.2) is given. When the shade of colour varies according to the samples, two or more reference solutions of the same degree of colour may be mentioned, or even only the degree of coloration without specifying the actual colour.

For material intended for parenteral use and for highly coloured solutions, especially when the use of Method I is contemplated, it is preferable to apply a limit of absorbance measured with a spectrophotometer at a suitable wavelength (usually between 400 and 450 nm). The concentration of the solution and the limit of absorbance must be stated. The conditions and limit must be based on knowledge of the absorbance curve in the range 400 to 450 nm and on results obtained with appropriate samples, including stored and degraded samples, as necessary.

2.4.5 pH or Acidity/Alkalinity

This test allows the limitation of acidic or alkaline impurities stemming from the method of preparation or purification or arising from degradation (e.g. from inappropriate storage) of the substance. The test may also be used to verify the stoichiometric composition of certain salts.

Two types of test for protolytic impurities are used in the Pharmacopoeia: a semi-quantitative titration experiment using indicators or electrometric methods to define the limits, the acidity-alkalinity test; or a pH measurement.

pH measurement is included if the material has buffering properties, otherwise a titrimetric procedure is recommended.

The question of whether to prescribe an acidity-alkalinity test or a pH measurement in a Pharmacopoeial monograph can be decided on the basis of an estimation of the buffering properties of the material. To this end a titration curve can be constructed for an aqueous solution (or, if necessary, extract) in the intended concentration (10 to 50 g/l) of a, preferably pure, specimen of the substance to be examined, using 0.01 M hydrochloric acid and 0.01 M sodium hydroxide, respectively and potentiometric pH measurement.

The inflexion point of the titration curve is the true pH of the solution and will, for a pure substance, be at the point of intersection with the pH-axis. The measure of the buffering capacity of the solution to be examined is the total shift in pH, (ΔpH), read from the titration curve as the result of adding on the one hand 0.25 ml of 0.01 M sodium hydroxide to 10 ml of the solution, and on the other hand 0.25 ml of 0.01 M hydrochloric acid to another 10 ml portion of the same solution. The larger ΔpH is, the lower is thus the buffering capacity. For a sample that is not quite pure, carry out a parallel displacement of the titration curve so that the true pH of the solution is on the pH-axis before the ΔpH is read from the curve.

The magnitude of ΔpH of the solution to be examined determines the choice of method for the limitation of protolytic impurities according to the following scheme. The classification is based upon the observation that the colour change for most indicators takes place over a pH range of 2 units.

Class A	ΔpH > 4	Acidity-alkalinity test utilising two appropriate indicators.
Class B	4 > ΔpH > 2	Acidity-alkalinity test utilising a single appropriate indicator.
Class C	2 > ΔpH > 0.2	A direct pH measurement.
Class D	ΔpH < 0.2	The protolytic purity cannot be reasonably controlled. substances that are salts consisting of ions with more than one acidic and/or basic function belong to this class and for these a pH measurement can contribute to ensuring the intended composition if the limits are sufficiently narrow

It is evident that by changing the concentration of the solution to be examined the class of buffering properties as set out above into which the substance will fall can to some extent be altered, since the shape of the titration curve will then also be modified. The concentration range given above is not to be exceeded, however, unless poor water solubility makes it unavoidable to use a more dilute solution.

In certain cases a test for acidity-alkalinity cannot be performed with the use of indicators due to coloration of the solution to be examined or other complications, and the limits are then controlled electrometrically. If on the other hand, the addition of standard acid/or base results in decomposition or precipitation of the substance to be examined, it may be necessary regardless of the buffering properties to prescribe a pH test.

If, for special reasons, as mentioned above, a pH measurement has to be prescribed for solutions with little or no buffering capacity, the solution to be examined is prepared with carbon dioxide-free water. Conversely, the use of carbon dioxide-free water for preparing solutions that have sufficient buffering capacity to warrant a direct pH measurement is not necessary since the required precision, which seldom exceeds one-tenth of a pH unit, will not thereby be affected. When an acidity requirement corresponds to not more than 0.1 ml of 0.01 M sodium hydroxide per 10 ml of solution to be examined the latter must be prepared using water free from carbon dioxide. These considerations are to be borne in mind when prescribing the composition of solution S if it is to be used in a test for protolytic impurities.

2.4.6 Optical Rotation

The optical rotation test, though sometimes useful for identification purposes, is mainly used as a purity test;

- either to assess the general purity of an optically active substance (a liquid or a solid in solution), by calculating the “Specific optical rotation” (title of the test), or
- to limit the presence of optically active impurities in any optically inactive substance (racemate or racemic mixtures), provided that the specific optical rotation at 589.3 nm is sufficient to ensure adequate sensitivity. In this case the range normally given should be +0.10° to - 0.10° (covering the substances which are not true racemates). In this case the “Angle of rotation” (title of the test) of the liquid or of a solution of the solid, is measured under defined conditions.

It is usually more appropriate to control these impurities by chiral separation methods since the specific optical rotation is often insufficient to limit the presence of the unwanted enantiomer (distomer) in the presence of the active enantiomer (eutomer).

The test is not suitable for highly coloured or opalescent solutions (in the latter case a filtration can sometimes make the determination possible).

The following aspects are taken into account in describing the test:

- the solvent, which depends on the solubility of the substance to be examined and the rotatory power in that solvent. In the case of non-aqueous solvents their purity and especially their contents of water must often be carefully defined;
- the concentration of the solution; it must be high enough to give a reliable reading of the angle of rotation;
- the quantity of substance to be used, determined with sufficient relative precision (generally 1 per cent), as is also the volume to be obtained (given with one decimal figure);
- the volume required which depends on the apparatus used, but since it rarely exceeds 25.0 ml that volume is usually prescribed;
- the degree of hydration or organic solvation of the substance must be taken into account in calculating the result;
- the result is the mean of at least 5 measurements when evaluated visually, with an apparatus giving readings to the nearest 0.01°;
- measured angles of rotation (rarely more than 2°) are given to two decimal places;
- specific optical rotation values are given with two or three significant figures. Values under 10° are given with two significant figures, while values of 10° and over are given with three significant figures.
- composition limit for racemates or racemic mixtures.

2.4.7 Absorption spectrophotometry (ultraviolet and visible)

The absorption of electromagnetic radiation may be used in purity tests as a limit test for certain impurities. The typical case is that of impurities that absorb in a region where the substance to be examined is transparent. Then it is the absorbance of a solution of the substance to be examined that is measured. This test may be performed in the following ways;

- by direct measurement on the solution, where the absorbance measured is a maximum absorbance at a given wavelength, or over a wavelength range,
- after carrying out a chemical reaction that forms, with the impurity, a substance that absorbs at a wavelength where the substance to be examined is transparent, a maximum value at the given wavelength being prescribed.

For measurements in the ultraviolet, it is advisable not to measure below 230 nm.

It is important to describe precisely the operational conditions to be observed, in particular the preparation of those solutions that are prepared by successive dilutions.

2.4.8 Related Substances

The policy on control of impurities is described in general chapter *5.10 Control of impurities in substances for pharmaceutical use* and in the general monograph *Substances for Pharmaceutical Use (2034)*. Monographs should be elaborated accordingly. Monographs are designed to take account of substances used in approved medicinal products in Member States and should provide adequate control of all impurities occurring in these substances, insofar as the necessary information and samples (substance and impurities) are available from the producers. Where the required information and samples are not provided for a substance synthesised by a given method, the monograph will not necessarily cover the corresponding impurity profile.

The provisions for related substances of the general monograph *Substances for Pharmaceutical Use (2034)* apply to all active substances covered by a monograph, unless otherwise stated. The following are given as exceptions in the general monograph:

— biological and biotechnological products, peptides, oligonucleotides, radiopharmaceuticals, products of fermentation and semi-synthetic products derived therefrom, to crude products of animal or plant origin or herbal products.

If an exception is to be made for some other substance, a statement is included in the specific monograph:

"The thresholds indicated under 'Related substances' (Table 2034.-1) in the general monograph *Substances for Pharmaceutical Use (2034)* do not apply."

Monographs should include acceptance criteria for:

- each specified impurity;
- unspecified impurities (previously referred to as "any other impurities"), normally set at the identification threshold;
- total impurities.

Impurities to be controlled include: intermediates and by-products of synthesis; co-extracted substances in products of natural origin; degradation products. Monographs on organic chemicals usually have a test entitled 'Related substances' (or a test with equivalent purpose under a different title), designed for control of organic impurities. Inorganic impurities are usually covered, where applicable, by other tests. Residual solvents are covered by specific provisions [see below and *5.4 Control of residual solvents*, *Substances for Pharmaceutical Use (2034)*].

The most common and the preferred method for control of organic impurities is liquid chromatography; gas chromatography or capillary electrophoresis may be the preferred method in some instances. Although many existing monographs apply thin-layer chromatography, in future this method should be reserved for control of specific impurities that cannot be conveniently be controlled by LC or GC. Existing TLC tests that do not follow this recommendation will be gradually replaced as soon as information on suitable LC or GC tests becomes available.

Monographs frequently have to be designed to cover different impurity profiles because of the use of different synthetic routes and purification procedures by producers. The usual

practice is to include a general LC test, supplemented where necessary by other tests (LC, GC, CE, TLC, or other techniques) for specific impurities. It is however becoming increasingly impractical in some cases to design a single general test and in such cases more than one general test is included and the scope of the different tests is defined in the tests themselves with a cross-reference in the Impurities section.

Monographs cover a number of specified impurities designated in the Impurities section. Specified impurities are those that occur in current batches of the substances used in approved products and for which an individual acceptance criterion is provided. Wherever feasible, monographs also have an acceptance criterion for other impurities (at the identification threshold for the substance) and a limit for the total of impurities (or a limit for the total of impurities other than a number of identified specified impurities) above the reporting threshold. The acceptance criterion for specified impurities may be set at the identification threshold for the substance.

The acceptance criteria for specified impurities take account of both:

1. qualification data, where applicable, the limit being set at a level not greater than that at which the impurity is qualified; the information on qualification is provided by the producer and the compatibility of the limit with the qualification data and approved specifications is checked by the competent authorities during elaboration of the monograph and/or during the Pharmeuropa comment phase; and
2. batch analysis data, the acceptance criteria being set to take account of normal production; data is provided by the producer for typical batches and checked during elaboration of the monograph on not fewer than 3 batches.

Separation methods For pharmacopoeial purposes the objective of a purity test using a separation method will usually be the control of impurities derived from one or more known manufacturing processes and decomposition routes. However, the experimental conditions are chosen for the test, especially the detection system, so as not to make it unnecessarily narrow in scope. Chromatographic purity tests may often be the best means of providing a general screening of organic impurities derived from new methods of manufacture or accidental contamination. It may be advantageous to supplement a chromatographic test with other chromatographic or non-chromatographic tests.

As mentioned in the section concerning identification, a chromatographic system applied to purity testing may, when suitable, be applied also for identification.

When a related substances test, based on a chromatographic technique is carried out, a representative chromatogram is published with the monograph in Pharmeuropa.

When no individual impurity is available as a reference substance or when a large number of impurities may be detected in the substance, a representative chromatogram will be supplied with the reference substance.

Monographs should provide a reliable means of locating all specified impurities on the chromatogram. Identification of unspecified impurities is necessary if a correction factor is to be applied. Peaks may be located using:

— a reference standard for each impurity;

— a reference standard containing some or all of the specified impurities, provided with a chromatogram.

Location by relative retention is not generally considered sufficient for pharmacopoeial purposes, notably for gradient elution.

Where a reference standard containing a mixture of impurities is to be used, a sample of each specified impurity should be provided to EDQM to enable the establishment of the reference standard.

General considerations applying to separation techniques:

- high concentration/loading are normally used since the symmetry of the principal peak or shape of the spot is not critical in impurity testing so long as there is no interference. When using an external standard in quantitative determinations then the response of the principal peak need not be in the linear range of the detector;
- in general tests for related substances, the substance to be examined should not to be chemically modified (eg derivatisation) before purity testing since the impurity pattern may be modified;
- similarly, extraction of the free base or acid prior to impurity testing is to be avoided.

2.4.8.1 Thin-layer Chromatography (TLC)

Thin-layer chromatography methods should only be used for control of a specified impurity and where liquid chromatography, gas chromatography or capillary electrophoresis methods are inappropriate (usually due to a lack of a suitable detection system).

Commercially available pre-coated plates, described in the reagents section, are to be used; the trade name of the plate found suitable is indicated in a footnote to the draft monograph, and posted in the 'knowledge' database on the EDQM web site after adoption of the monograph. In the reagents section, besides information on the coating material used (type of coating material, type of binder), a suitability test procedure is described. The monograph must describe the type of plate and include a system suitability requirement . Often the substances that would be best suited for a system suitability test will not be readily available individually; then a sample of the substance to be examined containing them as contaminants or even a deliberately spiked sample may be prescribed. Permissible variations to the different parameters are indicated in general chapter *2.2.46 Chromatographic separation techniques*.

If any pre-treatment is required or if the chromatography is carried out in unsaturated conditions for the satisfactory conduct of the test, then this information is included in the text of the monograph (especially applicable to the use of reverse phase plates).

One or more dilutions of the substance to be examined will often prove adequate for reference purposes, provided the impurities that are to be compared exhibit a similar behaviour under the chosen chromatographic conditions. This implies that the spots to be compared must be sufficiently close in Rf value to minimise errors introduced by different diffusion of the substances during their migration. Otherwise, reference solutions containing the specified impurities are to be employed. It may be necessary to instruct the analyst to

disregard a spot - often due to the non-migrating counter-ion of a salt - remaining on the starting line.

Summation of the responses exhibited by each individual spot is only acceptable when appropriate equipment is prescribed. It is not recommended to set a limit or limits for the concentration of impurities without a limit on their number, otherwise the total theoretical impurity level would be unacceptably high. This situation may be counteracted by limiting the impurities on two or more levels, allowing only a defined number to be at the higher level and the rest below the lower level. As examples, the test may specify that no contaminant may exceed a relative concentration of 1 per cent and that only one may exceed 0.25 per cent or that no contaminant may exceed a relative concentration of 1 per cent, only one contaminant above 0.5 per cent and no more than 4 contaminants above 0.25 per cent.

2.4.8.2 Liquid Chromatography (LC)

Defining the appropriate chromatographic system will often be one of the major problems to be dealt with in elaborating a pharmacopoeial purity test based on LC. In LC the matter is further complicated, however, by the existence of numerous variants of stationary phases, especially amongst the chemically bonded reverse phase materials for which not only brand to brand but occasionally also batch to batch variations occur that can influence a given separation. The trade name of the stationary phase/column(s) found suitable during elaboration of the monograph is indicated in a footnote to the draft monograph and transferred to the EDQM web site Knowledge database after adoption of the monograph.

Validation requirements are given in Section 3. The following are to be investigated:

- specificity,
- limit of detection,
- limit of quantification,
- precision,
- response factors (of the individual impurities),
- linearity (over the range of interest for a related substances test).

In describing the chromatographic system, mention must be made of the column dimensions (length and internal diameter), nature of the stationary phase (in detail) including any steps to prepare or pre-treat it, composition and flow rate of the mobile phase including elution programme (if any), column temperature (if differing from ambient or especially if thermostatted), method of injection (if important), injection volume and method of detection. Permissible variations to the different parameters are indicated in general chapter *2.2.46 Chromatographic separation techniques*.

When the separation has been found to be satisfactory on only one type of stationary phase tested, the latter must be well described, for example including the following information: type of particles (irregular or spherical), the particle size, the specific surface area (m^2/g) the pore size (nm) and when using reverse-phase columns the extent of carbon loading (per cent) and indicate whether the stationary phase is end-capped or otherwise treated to inactivate the residual silanol groups (this is particularly important when basic substances are to be examined and there is a risk of peak tailing).

Test and reference solutions are wherever possible prepared using the mobile phase as the solvent in order to minimise peak anomalies.

For the sake of simplicity and reproducibility, isocratic elution is to be preferred and the chromatography is carried out at normal room temperature (18 °C to 22 °C). Where a different temperature is advantageous, the temperature is specified (≥ 30 °C). When a gradient system is described, all necessary parameters must be clearly given e.g. composition of mobile phases, equilibrium conditions, gradient conditions (linear or step) etc.

Since many active pharmaceutical substances are synthesised by a number of synthetic routes the list of potential impurities to be limited may be large and the analytical challenge to separate them is great. Isocratic liquid chromatographic methods may not be sufficiently selective so that there is an increasing need to employ gradient methods. Thus, it is important to be aware of the potential pitfalls of significant differences in dwell volume in the context of method transfer.

When consideration is given to gradient elution in liquid chromatography an important parameter to be considered is the volume between the solvent mixing chamber and the head of the column. This volume is sometimes referred to as the dwell volume, V_D (other terms employed include: effective system delay volume, dead volume and delay volume). Large differences in dwell volume from one pumping system to another will result in differences in elution of peaks. The dwell volume is dependent on the configurations of the pumping system including the dimensions of the capillary tubing, the solvent mixing chamber and the injection loop; it is constant for a particular system. The greatest effect of differing dwell volumes on retention times is for those substances that are not strongly retained. Thus, gradient systems should be conceived in such a way that analytes are not eluted at the beginning of a gradient. It is best if less strongly retained components are eluted with an initial isocratic phase followed by a gradient for elution of the more strongly retained analytes. The effect of differences in dwell volumes is then minimised. In addition, an initial isocratic phase allows to correct for marked differences in dwell volume from one gradient pumping system to another.

It seems that the specification for the dwell volume whether for high pressure or low pressure gradient pumping systems are similar (all less than 1.0 ml). However, the dwell volume must be experimentally determined. If the measured dwell volume is 1.0 ml or less there is little difference in retention time or relative retention between different systems.

In conclusion it is recommended that whenever possible isocratic liquid chromatography be employed but if gradient elution is unavoidable then:

— the characteristics of the stationary phase employed should be described in detail;

— the gradient elution should be preceded by an isocratic step to elute the less retained analytes;

— the gradient elution profile should be such that elution of the analytes does not occur at or near the beginning of the gradient;

— the dwell volume of the pumping system employed to develop the method should be less than 1.0 ml.

If the method is developed using a system with a dwell volume greater than 1.0 ml, then a suitable initial isocratic step is essential.

During the validation of the method several types of stationary phase should be tested and the names of materials found to be suitable indicated in a footnote to the monograph published in Pharmeuropa.

2.4.8.2.1 System suitability criteria

One or more system suitability criteria are to be included in the test. Requirements in *2.2.46 Chromatographic separation techniques* are also applicable.

Separation capacity

Such a criterion is necessary when separation techniques are employed for assays and test for related substances. The following approaches most of which require the separation or partial separation of a critical pair, are acceptable for a system suitability test for selectivity:

—**Resolution.** As calculated by the formula given in the general text (2.2.29) using two closely eluting peaks, preferably corresponding to the substance itself and a potential impurity. However, when the elution times of the two peaks are very different i.e. the resolution factor is large (> 5.0) the use of the resolution factor as a performance test has little value. It is preferable to use another impurity or another substance chemically related to the substance under study, giving a smaller resolution factor. Peaks of different heights may be used for calculation of resolution but extreme differences will compromise the usefulness of the criterion.

—**Peak-to-valley ratio.** Can be employed when complete separation between two adjacent peaks cannot be achieved i.e. the resolution factor is less than 1.5.

In-situ degradation offers an alternative approach to define the suitability of the system provided that the solution of the substance can be degraded, in mild 'stress' conditions within a reasonably short time, to produce decomposition products, the peaks of which can be used to determine a resolution or a peak-to-valley ratio.

Chromatogram of a 'Spiked' or an Impure Substance. Can also be employed to define the system. This approach can be employed when it is difficult to isolate an impurity eluting close to the main peak in sufficient quantity to establish a reference substance. In this case a chromatogram can be supplied with the reference substance (for system suitability), or published with the monograph or described in the text of the test for related substances.

The use of a spiked or impure substance requires procurement of sufficient material to establish the reference substance used and in the future replacement of the system suitability test material with material exhibiting the same characteristics.

The methods of choice for defining the performance of the system, are the calculation of the resolution and the peak-to-valley ratio and such a requirement is also to be included when using a CRS of a 'spiked' or impure substance. When gradient elution is described it is preferable to describe a system suitability requirement for each critical gradient step.

It should be noted that the inclusion of retention times or relative retention values are given only for identification of peaks and do not constitute alternative system suitability criteria.

2.4.8.2.2 Quantification

Quantification is required for limits applied to specified impurities, unspecified impurities and total impurities. It is most commonly achieved using an external standard and less commonly by the normalisation procedure. Where a limit for total impurities is stated, a disregard limit should be defined, usually at the reporting threshold [see *Substances for Pharmaceutical Use (2034)*]; a dilution of the test solution is usually used to set the disregard limit.

External standard. A dilution of the test solution/substance to be examined is used, unless there is a large difference in the detector response of a specified (or exceptionally an unspecified) impurity that necessitates the use of a specific external standard, which may be:

— a solution of the impurity (preferred option)

— a solution of the substance to be examined containing a known amount of the impurity

Where a dilution of the substance to be examined is used as external standard, if the detector response differs by more than 20 per cent from that of the substance to be examined, a correction factor is indicated in the monograph.

Normalisation procedure. Quantification by the area normalisation technique requires that all the solutes are known to be eluted and detected, preferably with uniform response factors, and that the detector response is linear with the concentrations employed. This must be validated.

2.4.8.3 Gas-Liquid Chromatography (GC)

The difficulties met when defining the appropriate chromatographic system are similar in GC purity tests to those mentioned under LC although the emphasis may be on other points. The experimental details to be described in a pharmacopoeial test must, therefore, also here be worded as an example so that the chromatographic parameters may be varied to obtain the required performance. The nature of the stationary phase, i.e. the composition of the coating material (including its concentration) and the inert support (including its particle size and any pre-treatment) must also be given here in general terms but the details are to be recorded for subsequent publication in PHARMEUROPA.

In describing the chromatographic system mention must be made of essentially the same factors as mentioned under LC with appropriate variations, e.g. temperature programme (if any) instead of elution programme, injection port and detector temperatures etc. Use of packed columns should be avoided. Permissible variations to the different parameters are indicated in general chapter *2.2.46 Chromatographic separation techniques*.

For the sake of simplicity and reproducibility isothermal operating conditions are preferred. Quantification is usually based on an internal standard technique or on the area normalisation procedure. The same limitations concerning summation of peak responses as mentioned for LC apply here as well.

2.4.8.4 Capillary Electrophoresis

Capillary electrophoresis is increasingly employed to separate and control a large number of impurities of vastly different polarities. It is also suitable to control the content of the unwanted enantiomer in chiral therapeutic substances. Where the separation is conducted in a fused-silica capillary the problem, encountered in reversed-phase liquid chromatography, of varying performance from different stationary phases is avoided.

Joule heating occurs during a run and to obtain satisfactory reproducibility a defined temperature is maintained using a thermostat; for instruments without a thermostat, a low voltage should be used.

The limit of detection is adversely affected by the small injection volume and the small detection pathway in the capillary, even when stacking techniques are applied. For the control of impurities or assays the use of an internal standard is recommended to achieve appropriate precision. Otherwise the guidance for the use of this technique is similar to that given previously for liquid chromatography.

For chiral analysis, a chiral reagent is added to the running buffer. The chiral reagent should be carefully described in the monograph or as a reagent., particularly for cyclodextrin derivatives. Since many of the cyclodextrin derivatives are randomly substituted it is important to give the precise or average degree and location of substitution. During validation of the method more than one batch of the cyclodextrin derivative should be used.

Experimental parameters to be considered for inclusion in the monograph:

- Instrumental parameters: voltage, polarity, temperature, capillary size (diameter and length - total and effective to the detector);
- Coating material of the capillary (where applicable);
- Buffer: pH, molarity, composition:
- Sample solvent
- Separation: pole outlet, U, I
- Injection: t, $U/\Delta p$
- Detection: wavelength, instrumentation
- Temperature
- Shelf-life of solutions
- Rinsing procedures (time, reagents, Δp) needed to stabilise the migration times and the resolution of the peaks:
- Pre-conditioning of a new capillary
- Pre-conditioning of the capillary before a series of measurements
- Between-run rinsing

As a footnote for transfer to the EDQM web site knowledge database after publication of the monograph:

- if a coated capillary is used, the trade name of the capillary found suitable during elaboration of the monograph;
- for chiral separations, the trade name of the chiral reagent (cyclodextrin or other) found suitable during elaboration of the monograph.

In order to minimise the EOF signal, test and reference solutions are wherever possible prepared using water for injections or the running buffer as the solvent.

2.4.9 Readily Carbonisable Substances

The value of this non-specific test has greatly diminished through the introduction of chromatographic tests providing more information on organic impurities. The major advantage of a test for readily carbonisable substances is its often high sensitivity, if required. On the other hand, practice has shown that the impurities which produce a coloration under the conditions of the test often will respond equally well towards a test for colour in simple aqueous or alcoholic solution, and in such cases unnecessary duplication is to be avoided.

If, during the development of a monograph, it appears that impurities may be present which are not accounted for by other tests, then this test is carried out and, if appropriate, included in the monograph.

2.4.10 Foreign Anions and/or Cations

Since strong inorganic acids and bases are widely used in syntheses, the contents of foreign anions and/or cations in a substance can be indicative of the extent to which it has been purified. They can also reveal whether contamination with closely related substances has taken place. On the other hand, the usually ionic impurities can often be removed from poorly water-soluble substances by treatment with water without necessarily removing the organic impurities. Tests for anions and cations therefore cannot replace a test for related substances in organic substances but they may constitute a useful supplement in the case of the water-soluble organic substances. For inorganic substances, which are usually prepared from other inorganics, a much broader range of tests for foreign ions must be contemplated.

Where the introduction of tests for foreign anions in organic substances is considered then a single one, either for chlorides, sulphates or - less commonly - nitrates, will usually suffice even when several could theoretically be present. The test is then to be carried out on the most abundant anion.

Certain cations must be stringently limited because of their toxicity or catalytic activity. They are treated separately below under heavy metals. Unless there are special reasons for limiting the presence of cations, individually or in smaller groups, in organic substances, the majority are adequately controlled via a determination of the sulphated ash (see later).

2.4.11 Heavy Metals

The heavy metals detected by the general methods are those that precipitate at pH 3.5 in the form of dark-coloured sulphides through the action of sulphide ions or reagents capable of producing them: lead, copper, silver, mercury, cadmium, bismuth, ruthenium, gold, platinum, palladium, vanadium, arsenic, antimony, tin and molybdenum (*Pharmeuropa*, Vol. 1, p 249). Thioacetamide is used as the precipitating agent and sodium sulphide is allowed as an

alternative but its suitability has to be demonstrated by the user for each substance using monitor solutions.

Comparison is made with a standard containing a known quantity of lead. The total "heavy metals" content is thus expressed in the form of lead, though the sensitivity for individual metals is different.

The Pharmacopoeia provides a choice between seven different methods:

- Methods A and B are based on the direct use of a solution in water or in an organic solvent. These methods are applicable to substances soluble under these conditions. If the solution is sufficiently clear and colourless (colour equal to or less intense than degree 6) the test can be carried out with comparison of the reaction solutions; otherwise, filtration and comparison of the filter membrane should be prescribed. The methods are applicable only if the substance does not either interfere with the precipitation of sulphides by the thioacetamide reagent or mask the metals by chelation; lack of interference must be verified by recovery experiments during validation of the test for inclusion in the monograph. Monitor solutions are not prepared for methods A and B, although if sodium sulphide is used as precipitating agent then the suitability has to be demonstrated initially by preparing monitor solutions as defined in the general method.
- Method E has a lower limit of applicability (2 µg of lead in a 30 ml volume of solution). A monitor solution is included.
- Methods C, D, F and G include prior digestion. They are applied where methods A and B are not feasible (lack of solubility, chelating properties, interference with the precipitation of metal sulphides by the thioacetamide reagent). Methods C and D lack robustness since a number of heavy metals will be lost to varying degrees by volatilisation during the digestion, for example mercury, lead and arsenic. Methods F and G, which use "wet" digestion, are now preferred. Method F is time-consuming; method G, using microwave-assisted digestion, is more rapid and convenient. Monitor solutions are included for these methods.

In routine practice, Methods A, B, C, D, F and G are unsuitable for establishing limits below 5 ppm, unless filtration is prescribed. For lower limits, Method E can be used, which makes it possible to go down to 0.5 ppm. In order to ensure limit contents of less than 0.5 ppm, it is necessary to resort to tests specific for each metal, which are frequently based on atomic spectrophotometry.

Criteria for inclusion of a heavy metals test:

Daily intake > 0.5 g/day, treatment < 30 days	heavy metals test, limit 20 ppm
Daily intake > 0.5 g/day, treatment > 30 days	heavy metals test, limit 10 ppm
Daily intake < 0.5 g/day, treatment > 30 days	heavy metals test, limit 10 ppm if the substance is used parenterally, otherwise 20 ppm
Daily intake < 0.5 g/day, treatment < 30 days	no heavy metals test,

Tests for individual metal catalysts are included where it is known that they are used in the synthesis of an active substance or excipient. Limits take account of toxicity, the route(s) of administration and the manufacturing process.

2.4.12 Loss on drying

Generally an upper limit for loss on drying is given. If the substance is a hydrate (or solvate) upper and lower limits are indicated. Drying is carried out to constant mass, unless a drying time is specified in the monograph. When a drying time is prescribed, adequate validation data must be provided. Where the drying temperature is indicated using a single value, a tolerance of ± 2 °C is understood. For temperatures higher than 105 °C, a larger tolerance should be indicated in the monograph, if necessary.

The general chapter includes five sets of standard conditions that are referred to in monographs using conventional expressions:

a) "in a desiccator" (over P_2O_5 at atmospheric pressure and at room temperature);

b) "*in vacuo*" (over P_2O_5 at 1.5-2.5 kPa at room temperature);

c) "*in vacuo* within a specified temperature range" (over P_2O_5 at 1.5-2.5 kPa within the temperature range specified in the monograph);

d) "in an oven within a specified temperature range" (temperature specified is preferably 105 °C (for harmonisation with JP and USP), a tolerance of ± 2 °C being implied);

e) "under high vacuum" (over P_2O_5 at ≤ 0.1 kPa at the temperature prescribed in the monograph);

If other conditions are used, they are described in full in the monograph.

Limits less than 10 per cent should be given with 2 significant figures and limits of 10 per cent or greater should be given with 3 significant figures. The sample size is chosen to give a difference of 5-50 mg before/after drying and is indicated with 4 significant figures.

The test can be carried out on a semi-micro scale, in which case the accuracy with which the test sample is to be weighed should be specified accordingly.

Method d) is to be preferred when the product is sufficiently stable at 105 °C. Otherwise, a method b) or c) is usually applied. It must however be remembered that organic solvents are not always easily removed (e.g. organic solvents in colchicine).

2.4.13 Thermogravimetry (2.2.34)

Loss on drying can be determined by this method when the amount of substance has to be restricted, for example to reduce exposure for the analyst or if the substance is very expensive (e.g. vincristine sulphate and vinblastine sulphate).

2.4.14 Semi-micro determination of water (Karl Fischer – 2.5.12)

Commercial reagents without pyridine are now used instead of *iodosulphurous reagent R*; stoichiometry and freedom from interference are to be verified (data may be provided by the supplier of the reagent for the substance in question).

The commercial name of the titrant used during development of the monograph should be indicated in a footnote to the monograph; it will be transferred to the EDQM Knowledge database after adoption of the monograph.

Limits less than 10 per cent should be given with 2 significant figures and limits of 10 per cent or greater should be given with 3 significant figures. Semi-micro determination is not recommended for a water content less than 0.5 per cent. The sample size is chosen to obtain a titration volume of about 1 ml and should be given with 3 significant figures.

2.4.15 Micro determination of water (2.5.32)

In the General Method no detailed description is given for the composition of the electrolyte (anolyte and catolyte) reagent, as almost all laboratories use commercially available ready-to-use reagents.

Limits should be expressed with 2 significant figures. The sample size is chosen to have a water content of 10 µg to 10 mg; titration of quantities of the order of 10 µg are prescribed only where the water content is very low or the sample size is limited by the cost of the substance. The sample size should be stated with 3 significant figures.

2.4.16 Gas Chromatographic determination of water

Method may also be used for the determination of water.

2.4.17 Azeotropic Distillation (2.2.13)

This method is used mainly for herbal drugs. It is applicable to a quantity of substance capable of yielding 2 to 3 ml of water.

2.4.18 Sulphated Ash

This test is usually intended for the global determination of foreign cations present in organic substances and in those inorganic substances which themselves are volatilised under the conditions of the test. Thus the test will be of little value as a purity requirement for the majority of inorganic salts of organic substances, due to the resulting high bias.

The limit in a test for sulphated ash is usually set at 0.1 per cent, unless otherwise justified. The amount of substance prescribed for the test must be such that a residue corresponding to the limit will weigh not less than 1.0 mg and the prescribed mass of substance is then given with the appropriate precision (1.0 g).

2.4.19 Residue on Evaporation

The amount of a liquid material prescribed for the test is such that a residue corresponding to the limit will weigh at least 1.0 mg. The appropriate mass or volume of the substance will normally be in the range of 10 g to 100 g (or ml).

2.4.20 Residual Solvents

Control of residual solvents is provided for in general chapter *5.4 Residual solvents* and in the general monograph *Substances for Pharmaceutical Use (2034)*, which apply the ICH Guideline.

A test for a class 1 solvent is included in the monograph if it is potentially present in an approved product.

Tests for class 2 solvents are not included in monographs since the limit may be set using option 2 of chapter 5.4, whereby all the ingredients of a pharmaceutical preparation are taken into account.

A test for a class 3 solvent is included if it is potentially present in an approved product at a level higher than 0.5 per cent.

2.5 ASSAY

Assays are included in monographs unless:

— all the foreseeable impurities can be detected and limited with sufficient precision;

— certain quantitative tests, similar to assays, are carried out with sufficient precision (specific optical rotation, specific absorbance,....);

— specific profiles of relevant substances such as composition of the fatty acid fraction (2.4.22) or composition of the sterol fraction (2.4.23) of a fat or fatty oil have been established;

— the tests performed are sufficient to establish the quality of the substance, usually a non-active ingredient for example, ethanol and water.

In certain cases, more than one assay may be necessary when:

— the substance to be examined consists of a combination of two parts which are not necessarily present in absolutely fixed proportions, so that the assay of only one of the two constituents does not make it possible correctly to determine the substance as a whole (e.g. theophylline and ethylenediamine);

— the results of the quantitative tests do not fully represent the therapeutic activity, then a biological assay is included.

In the case of well-defined salts, the assay of only one of the ions, preferably the pharmacologically active component, is generally considered sufficient. It is only rarely necessary to determine all the ions and, in any case, it is considered superfluous to determine one of these by two methods even when these depend on different principles.

When the identification and purity tests are sufficiently characteristic and searching, a non-specific but precise assay may be used rather than a specific and less precise assay.

Every assay method proposed must be validated according to the procedures described for the different techniques in section 3.

2.5.1 Ultraviolet and Visible Spectrophotometry

Spectrophotometric assays may be carried out directly in the ultraviolet or visible range or after a suitable chemical reaction, though the latter are less precise. Other methods are usually preferred.

2.5.1.1 direct measurement

This is not specific but of acceptable precision and is usually performed without a reference substance: the absorbance of the solution is measured at the specified absorption maximum, and the content of the substance to be examined is calculated on the basis of the specific absorbance stated in the monograph.

The specific absorbance value must be verified:

— for a new substance, the manufacturer must supply validation data supporting the acceptance of the "true" value. Information supplied includes, for example, the purity of the substance used to determine the value. This is demonstrated by employing several methods, including separation techniques, absolute methods, the response factors of likely impurities, etc,

With a reference substance, the active principle content is calculated from a comparison between the absorbance of the solution to be examined and that of a solution of a reference substance.

For experimental details and results see "Ultraviolet Spectrophotometry" (2.2.25).

2.5.1.2 measurement after a colour reaction

This measurement is carried out by comparison with a reference substance. The results may be less precise due to manipulation.

2.5.2 Volumetric analysis

The amount of the substance taken for the assay is such that the final titration, using automatic titration equipment, will consume less than 10 ml – preferably between 7 and 8 ml – of titrant in order to permit the use of standard titration equipment. In the case of back-titration the fixed volume of the first titrant added must, furthermore, be adequate so that the result of the assay will not be based upon a small difference of volumes.

Blank tests are to be prescribed whenever necessary, unless already stipulated in the underlying general method.

Either a potentiometric end-point detection or a visual colour change indicator can be specified in the monograph. The potentiometric mode of end-point detection is clearly applicable in almost all cases and is to be preferred. Where potentiometric detection is specified, the appropriate combination of electrodes for that purpose is, whenever useful, to be given in the text. The number of inflexion points to be evaluated is given. Exceptionally, other modes of detection are specified, such as the amperometric method (*2.2.19*). Whichever mode is used, it must be known to be appropriately reproducible and preferably stoichiometrically exact. When a visual indicator is specified, the colour change is given only when it is different to that described in the Reagents chapter of the Pharmacopoeia.

Halide salts of organic bases and some quaternary ammonium substances have traditionally been determined by non-aqueous titration using perchloric acid in glacial acetic acid as the titrant and glacial acetic acid as the solvent with the addition of mercuric acetate. To avoid the use of mercury salts it is recommended that, wherever possible, other methods be employed. The following methods are recommended for consideration:

a. Alkalimetric titration in an alcoholic medium

When carrying out the alkalimetric titration it is advised to add 5 ml of 0.01 M hydrochloric acid before the titration and to measure the volume of titrant required between the two points of inflexion.

b. Titration with perchloric acid, the sample being dissolved in anhydrous acetic acid before addition of acetic anhydride or a mixture of acetic anhydride and anhydrous formic acid

c. Argentimetry

Methods b, and C are often suitable for quaternary ammonium substances.

2.5.3 Chromatography

The chromatographic methods on which assays may be based are in pharmacopoeial practice normally limited to liquid chromatography (LC) and gas chromatography (GC). The majority of the guidelines contained in the section on related substances for LC and GC will also be valid for elaborating assays based on these methods. The use of an external standard in LC and the addition of an internal standard in GC is recommended. Such methods require the use of a Chemical Reference Substance, which must be assigned a content of the analyte (see section on the Reference Standards).

When the method has been developed and validated by the author it is then necessary to assess its reproducibility (see VALIDATION).

2.5.4 Determination of Nitrogen by Sulphuric Acid Digestion (Semi-micro method)

Any substance to be assayed by this method has a digestion time assigned after a determination of its digestion profile.

The digestion profile may be determined in the following way. Several individually weighed portions of the prescribed amount of substance are assayed in accordance with the general method whilst varying the time for which the reaction mixture is boiled, normally up to 120 min, after the mixture has cleared. By plotting the resulting nitrogen content against the boiling time it is possible to determine the minimum digestion time necessary, to obtain constant values. In cases where the necessary digestion time exceeds 30 min, the time required is indicated in the monograph.

2.6 STORAGE

Although the statements given under this heading in a monograph of the Pharmacopoeia do not constitute pharmacopoeial requirements, the appropriate information to safeguard the quality of a pharmacopoeial material during storage is to be given here where appropriate.

The terminology to be found under 1. General Notices and in *3.2 Containers* should be used. Attention is drawn to the fact that the term “well-closed container” does not imply protection against loss or uptake of constituents via the gas phase but that the latter requires an “airtight container”. A “sealed container” is in effect “tamper-proof” at the same time, while the converse is not necessarily true.

Manufacturers should be requested to provide stability data. In considering the guidance to be given in the monograph, the behaviour of the material towards exposure to atmospheric air, various degrees of humidity, different temperatures and daylight are to be taken into account.

In this context it must be recalled that the method given in chapter *5.11 Characters section in monographs* for hygroscopicity is not to be used for defining storage conditions. This is a rapid method to give an indication of the hygroscopicity of the substance as an aid to the analyst so that the proper handling precautions can be taken when examining the substance in laboratory conditions.

2.7 LABELLING

In respect of the fact that the labelling of medicine is subject to international agreements and supranational and national regulations, the indications given under Labelling are not exhaustive: they consist of mandatory statements (necessary for the application of the monograph) and other statements which are included only as recommendations. In general, for bulk drug substances (active ingredients) the requirements given in this section of a Pharmacopoeial monograph is confined to those essential for the correct interpretation of the other requirements in the monograph. When, for example, a starting material has to comply with additional requirements (sterility, etc.) the label must state, where appropriate, that the contents of the container are suitable for that use. Furthermore, when the inclusion of certain stabilisers or other additives is authorised by the monograph, their presence will generally have to be declared on the label.

2.8 IMPURITIES

Monographs on organic chemicals should have an Impurities section defining the impurities that are known to be detected by the prescribed tests and that have been considered in defining the acceptance criteria for related substances. Subheadings are given for 'Specified impurities' and 'Other detectable impurities' All specified impurities covered by the monograph are included in the section. In addition, it may be useful to include information on other detectable impurities, (impurities that are known to be detected by the monograph tests but that are not known to occur in current production batches *above the identification threshold*).

The Impurities section gives a list showing for each impurity the chemical structure and chemical nomenclature (of the base/acid where applicable). Impurities are designated by a capital letter (A, B, C, D etc). Trivial names may be included in parenthesis in the rare cases where they are considered to be informative.

The Impurities section may also give information on the test(s) that limit(s) a given impurity, for example where this is not the related substances test or where there is more than one related substances test.

2.9 FUNCTIONALITY-RELATED CHARACTERISTICS

Monographs on excipients may have a section on functionality-related characteristics (FRCs). This is introduced by a standard paragraph indicating the non-mandatory status. The uses for which each FRC is relevant are also stated. FRCs may be presented:

- giving simply the name;
- giving the name and a recommended method from the general chapters of the Ph Eur;
- giving the name, a recommended method and recommended tolerances;
- giving the name, a recommended method, recommended tolerances and recommended acceptance criteria.

3 ANALYTICAL VALIDATION

This section describes the procedures to be carried out to validate the tests described in a monograph of the European Pharmacopoeia. These tests include tests for identification, instrumental and non-instrumental tests for the control of impurities and the assay method. The validation requirements vary according to the type of test and the technique employed. This section contains the texts on Analytical Validation adopted by the ICH in 1994, the Extension of the ICH text « Validation of Analytical Procedures » which includes valuable information concerning validation requirements for registration applications and specific guidelines for the validation of pharmaceutical methods using different analytical techniques.

3.1 DEFINITIONS AND TERMINOLOGY

Text adopted and published by International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (1994).

3.1.1 Introduction

This document presents a discussion of the characteristics for consideration during the validation of the analytical procedures included as part of registration applications submitted within the EC, Japan and USA. This document does not necessarily seek to cover the testing that may be required for registration in, or export to, other areas of the world. Furthermore, this text presentation serves as a collection of terms, and their definitions, and is not intended to provide direction on how to accomplish validation. These terms and definitions are meant to bridge the differences that often exist between various compendia and regulators of the EC, Japan and USA.

The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. A tabular summation of the characteristics applicable to identification, control of impurities and assay procedures is included. Other analytical procedures may be considered in future additions to this document.

3.1.2 Types of Analytical Procedures to be Validated

The discussion of the validation of analytical procedures is directed to the four most common types of analytical procedures:

- Identification tests;
- Quantitative tests for impurities' content;
- Limit tests for the control of impurities;
- Quantitative tests of the active moiety in samples of drug substance or drug product or other selected component(s) in the drug product.

Although there are many other analytical procedures, such as dissolution testing for drug products or particle size determination for drug substance, these have not been addressed in the initial text on validation of analytical procedures. Validation of these additional analytical

procedures is equally important to those listed herein and may be addressed in subsequent documents.

A brief description of the types of tests considered in this document is provided below.

- Identification tests are intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample (e.g. spectrum, chromatographic behaviour, chemical reactivity, etc.) to that of a reference standard.
- Testing for impurities can be either a quantitative test or a limit test for the impurity in a sample. Either test is intended to accurately reflect the purity characteristics of the sample. Different validation characteristics are required for a quantitative test than for a limit test.
- Assay procedures are intended to measure the analyte present in a given sample. In the context of this document, the assay represents a quantitative measurement of the major component(s) in the drug substance. For the drug product, similar validation characteristics also apply when assaying for the active or other selected component(s). The same validation characteristics may also apply to assays associated with other analytical procedures (e.g. dissolution).

3.1.3 Validation Characteristics and Requirements

The objective of the analytical procedure should be clearly understood since this will govern the validation characteristics which need to be evaluated. Typical validation characteristics that should be considered are listed below:

- Accuracy
- Precision
    - Repeatability
    - Intermediate Precision
- Specificity
- Detection Limit
- Quantitation Limit
- Linearity
- Range.

Each of these validation characteristics is defined in the attached Glossary. The table lists those validation characteristics regarded as the most important for the validation of different types of analytical procedures. This list should be considered typical for the analytical procedures cited but occasional exceptions should be dealt with on a case by case basis. It should be noted that robustness is not listed in the table but should be considered at an appropriate stage in the development of the analytical procedure.

Furthermore revalidation may be necessary in the following circumstances:

- changes in the synthesis of the drug substance;
- changes in the composition of the finished product;

- changes in the analytical procedure.

The degree of revalidation required depends on the nature of the changes. Certain other changes may require validation as well.

	TYPE OF ANALYTICAL PROCEDURE			
	IDENTIFICATION	**TESTING FOR IMPURITIES**		**ASSAY**
		Quantitative	Limits	Dissolution Measurement only Content / potency
CHARACTERISTIC				
Accuracy	-	+	-	+
Precision				
Repeatability		+	-	+
Intermediary Precision		+*	-	+*
Specificity**	+	+	+	+
Detection Limit	-	-***	+	-
Quantification Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- signifies that this characteristic is not normally evaluated.

+ signifies that this characteristic is normally evaluated.

* in cases where reproducibility (see glossary) has been performed, intermediate precision is not needed.

** lack of SPECIFICITY of one analytical procedure, could be compensated by other supporting analytical procedure(s).

*** may be needed in some cases.

3.1.4 Glossary

Analytical procedure. The analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: the sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

Specificity. Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s).

This definition has the following implications:

IDENTIFICATION to ensure the identity of an analyte.

PURITY TESTS to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte i.e. related substances test, heavy metals, residual solvents content, etc.

ASSAY (content or potency) to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

Accuracy. The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

This is sometimes termed trueness.

Precision. The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels; repeatability, intermediate precision and reproducibility.

Precision should be investigated using homogeneous, authentic samples. However, if it is not possible to obtain a homogeneous sample it may be investigated using artificially prepared samples or a sample solution.

The precision of analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Intermediate precision expresses within laboratories variations; different days, different analysts, different equipment, etc.

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardisation of methodology).

Detection limits. The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

Quantitation limits. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of substances in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

Linearity. The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Range. The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

Robustness. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

3.2 METHODOLOGY

[ICH document. Text adopted and published by the International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human use (1996)].

3.2.1 Introduction

This document is complementary to the parent document which presents a discussion of the characteristics that should be considered during the validation of analytical procedures. Its purpose is to provide some guidance and recommendations on how to consider the various validation characteristics for each analytical procedure. In some cases (for example, demonstration of specificity) the overall capabilities of a number of analytical procedures in combination may be investigated in order to ensure the quality of the drug substance or drug product. In addition, the document provides an indication of the data which should be presented in a new drug application.

All relevant data collected during validation and formulae used for calculating validation characteristics should be submitted and discussed as appropriate.

Approaches other than those set forth in this guideline may be applicable and acceptable. It is the responsibility of the applicant to choose the validation procedure and protocol most suitable for their product. However, it is important to remember that the main objective of validation of an analytical procedure is to demonstrate that the procedure is suitable for its intended purpose. Due to their complex nature, analytical procedures for biological and biotechnological products in some cases may be approached differently than in this document.

Well-characterised reference materials, with documented purity, should be used throughout the validation study. The degree of purity required depends on the intended use.

In accordance with the parent document and for the sake of clarity, this document considers the various validation characteristics in distinct sections. The arrangement of these sections reflects the process by which an analytical procedure may be developed and evaluated.

In practice, it is usually possible to design the experimental work such that the appropriate validation characteristics can be considered simultaneously to provide a sound, overall knowledge of the capabilities of the analytical procedure, for instance: specificity, linearity, range, accuracy and precision.

3.2.2 Specificity

An investigation of specificity should be conducted during the validation of identification tests, the determination of impurities and the assay. The procedures used to demonstrate specificity will depend on the intended objective of the analytical procedure.

It is not always possible to demonstrate that an analytical procedure is specific for a particular analyte (complete discrimination). In this case a combination of two or more analytical procedures is recommended to achieve the necessary level of discrimination.

3.2.2.1 identification

Suitable identification tests should be able to discriminate between substances of closely related structures which are likely to be present. The discrimination of a procedure may be confirmed by obtaining positive results (perhaps by comparison with a known reference material) from samples containing the analyte, coupled with negative results from samples which do not contain the analyte. In addition, the identification test may be applied to materials structurally similar to or closely related to the analyte to confirm that a positive response is not obtained. The choice of such potentially interfering materials should be based on sensible scientific judgement with a consideration of the interferences which could occur.

3.2.2.2 assays and impurity tests

For chromatographic procedures, representative chromatograms should be used to demonstrate specificity and individual components should be appropriately labelled. Similar considerations should be given to other separation techniques.

Critical separations in chromatography should be investigated at an appropriate level. For critical separations specificity can be demonstrated by the resolution of the two components which elute closest to each other.

In cases where a non-specific assay is used, other supporting analytical procedures should be used to demonstrate overall specificity. For example, where a titration is adopted to assay the drug substance, the combination of the assay and a suitable test for impurities can be used.

The approach is similar for both assays and impurity tests:

Impurities are available

- for the assay, this should involve demonstration of the discrimination of the analyte in the presence of impurities and/or excipients; practically, this can be done by spiking pure substances (drug substance or drug product) with appropriate levels of impurities and/or excipients and demonstrating that the assay result is unaffected by the presence of these materials (by comparison with the assay result obtained on unspiked samples);
- for the impurity test, the discrimination may be established by spiking drug substance or drug product with appropriate levels of impurities and demonstrating the separation of these impurities individually and/or from other components in the sample matrix. Alternatively, for less discriminating procedures it may be acceptable to demonstrate that these impurities can still be determined with appropriate accuracy and precision.

Impurities are not available

If impurity or degradation product standards are unavailable, specificity may be demonstrated by comparing the test results of samples containing impurities or degradation products to a second well-characterised procedure e.g.: pharmacopoeial method or other validated analytical procedure (independent procedure). As appropriate, this should include samples

stored under relevant stress conditions: light, heat, humidity, acid/base hydrolysis and oxidation.

- for the assay, the two results should be compared.
- for the impurity tests, the impurity profiles should be compared.

Peak purity tests may be useful to show that the analyte chromatographic peak is not attributable to more than one component (e.g. diode array, mass spectrometry).

3.2.3 Linearity

Linearity should be established across the range (see 3.2.4) of the analytical procedure. It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighings of synthetic mixtures of the drug product components using the proposed procedure. The latter aspect can be studied during investigation of the range.

Linearity should be established by visual evaluation of a plot of signals as a function of analyte concentration or content. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may have to be subjected to a mathematical transformation prior to the regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity. The correlation coefficient, y-intercept, slope of the regression line and residual sum of squares should be submitted. A plot of the data should be included. In addition, an analysis of the deviation of the actual data points from the regression line may also be helpful for evaluating linearity.

Some analytical procedures such as immunoassays do not demonstrate linearity after any transformation. In this case the analytical response should be described by an appropriate function of the concentration (amount) of an analyte in a sample.

For the establishment of linearity, a minimum of 5 concentrations is recommended. Other approaches should be justified.

3.2.4 Range

The specified range is normally derived from linearity studies and depends on the intended application of the procedure. It is established by confirming that the analytical procedure provides an acceptable degree of linearity, accuracy and precision when applied to samples containing amounts of analyte within or at the extremes of the specified range of the analytical procedure.

The following minimum specified ranges should be considered:

- for the assay of a drug substance or a finished product: from 80 to 120 per cent of the test concentration;
- for the determination of an impurity: from QL or from 50 % of the specification of each impurity, whichever is greater, to 120 % of the specification;

- for impurities known to be unusually potent or to produce toxic or unexpected pharmacological effects, the detection/quantitation limit should be commensurate with the level at which the impurities must be controlled.

Note: for validation of impurity test procedures carried out during development, it may be necessary to consider the range around a suggested (probable) limit;

- if assay and purity are performed together as one test and only a 100 % standard is used, linearity should cover the range from QL or from 50 % of the specification of each impurity, whichever is greater, to 120 % of the assay specification;
- for content uniformity, covering a minimum of 70 to 130 per cent of the test concentration, unless a wider more appropriate range, based on the nature of the dosage form (e.g. metered dose inhalers) is justified;
- for dissolution testing: ±20 % over the specified range; e.g. if the specifications for a controlled released product cover a region from 20 %, after 1 hour, up to 90 %, after 24 hours, the validated range would be 0-110 % of the label claim.

3.2.5 Accuracy

Accuracy should be established across the specified range of the analytical procedure.

3.2.5.1 Assay

Drug Substance

Several methods of determining accuracy are available:

- application of an analytical procedure to an analyte of known purity (e.g. reference material).
- comparison of the results of the proposed analytical procedure with those of a second well-characterised procedure, the accuracy of which is stated and/or defined (independent procedure);
- accuracy may be concurrently determined when precision, linearity and specificity data are acquired.

Drug Product

Several methods for determining accuracy are available:

- application of the analytical procedure to synthetic mixtures of the drug product components to which known quantities of the drug substance to be analysed have been added;
- in cases where it is impossible to obtain samples of all drug product components, it may be acceptable either to add known quantities of the analyte to the drug product or to compare the results obtained from the second, well-characterised procedure, the accuracy of which is stated and/or defined (independent procedure);
- accuracy may be concurrently determined when precision, linearity and specificity data are acquired.

3.2.5.2 Impurities (Quantification)

Accuracy should be assessed on samples (drug substance/drug product) spiked with known amounts of impurities.

In cases where it is impossible to obtain samples of certain impurities and/or degradation products, if is acceptable to compare results obtained by an independent procedure. The response factor of the drug substance can be used.

3.2.5.3 Recommended data

Accuracy should be assessed using a minimum of 9 determinations over a minimum of 3 concentration levels covering the specified range (e.g. 3 concentrations / 3 replicates each).

Accuracy should be reported as per cent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value together with the confidence intervals.

3.2.6 Precision

Validation of tests for assay and for quantitative determination of impurities, includes an investigation of precision.

3.2.6.1 repeatability

Repeatability should be assessed using:

- a minimum of 9 determinations covering the specified range for the procedure (e.g. 3 concentrations / 3 replicates each) *or*
- a minimum of 6 determinations at 100 % of the test concentration.

3.2.6.2 intermediate precision

The extent to which intermediate precision should be established depends on the circumstances under which the procedure is intended to be used. The applicant should establish the effects of random events on the precision of the analytical procedure. Typical variations to be studied include days, analysts, equipment, etc. It is not necessary to study these effects individually. The use of an experimental design (matrix) is encouraged.

3.2.6.3 reproducibility

Reproducibility is assessed by means of an inter-laboratory trial. Reproducibility should be considered in case of the standardisation of an analytical procedure, for instance, for inclusion of procedures in pharmacopoeias. These data are not part of the marketing authorisation dossier.

3.2.6.4 recommended data

The standard deviation, relative standard deviation (coefficient of variation) and confidence interval should be reported for each type of precision investigated.

3.2.7 Detection Limit

Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

3.2.7.1 based on visual evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods.

The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

3.2.7.2 based on Signal-to-Noise ratio

This approach can only be applied to analytical procedures which exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally acceptable.

3.2.7.3 based on the Standard Deviation of the Response and the Slope

The detection limit (DL) may be expressed as:

$$\mathrm{DL} = \frac{3.3\sigma}{S}$$

where σ = the standard deviation of the response.

where S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways, for example:

Based on the Standard Deviation of the Blank

Measurement of the magnitude of analytical background response is performed by analysing an appropriate number of blank samples and calculating the standard deviation of these responses.

Based on the Calibration Curve

A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

3.2.7.4 Recommended data

The detection limit and the method used for determining the detection limit should be presented.

In cases where an estimated value for the detection limit is obtained by calculation or extrapolation, this estimate may subsequently be validated by the independent analysis of a suitable number of samples known to be near or prepared at the detection limit.

3.2.8 Quantification Limit

Several approaches for determining the quantitation limit are possible, depending on whether the procedure is non-instrumental or instrumental. Approaches other than those listed may be acceptable.

3.2.8.1 based on visual evaluation

Visual evaluation may be used for non-instrumental methods, but may also be used with instrumental methods.

The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

3.2.8.2 based on Signal-to-Noise ratio

This approach can only be applied to analytical procedures which exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1.

3.2.8.3 based on the Standard Deviation of the Response and the Slope

The quantitation limit (QL) may be expressed as:

$$\mathrm{QL} = \frac{10\sigma}{S}$$

where σ = the standard deviation of the response

where S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate of the σ may be carried out in a variety of ways for example:

Based on the Standard Deviation of the Blank

Measurement of the magnitude of analytical background response is performed by analysing an appropriate number of blank samples and calculating the standard deviation of these responses.

Based on the Calibration Curve

A specific calibration curve should be studied using samples, containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

3.2.8.4 Recommended data

The quantitation limit and the method used for determining the quantitation limit should be presented.

The limit should be subsequently validated by the analysis of a suitable number of samples known to be near or prepared at the quantitation limit.

3.2.9 Robustness

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g. resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.

Typical variations are:

- stability of analytical solutions,
- different equipment,
- different analysts.

In the case of liquid chromatography, typical variations are:

- influence of variations of pH in a mobile phase,
- influence of variations in mobile phase composition,
- different columns (different lots and/or suppliers),
- temperature,
- flow rate.

In the case of gas-chromatography, typical variations are:

- different columns (different lots and/or suppliers),
- temperature,
- flow rate.

3.2.10 System Suitability Testing

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analysed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. See Pharmacopoeias for additional information.

3.3 SPECIFIC APPLICATION TO METHODS USED IN THE PHARMACOPOEIA

The following sections describe a number of points which are important for the validation of methods employing specific analytical techniques. These guidelines are to be used in conjunction with the general methods of the European Pharmacopoeia and the validation requirements given previously in the ICH documents.

3.3.1 Optical Rotation (2.2.7)

3.3.1.1 introduction

The solvent should be chosen in order to obtain an angle of rotation as high as possible. The stability of the angle of rotation of the solution should be checked over a period of at least 2 hours. If necessary, the use of a freshly prepared solution may be prescribed. In exceptional cases it might be necessary to prescribe an equilibration period before the measurement is carried out.

Whenever possible the use of the D-line of sodium is prescribed.

3.3.1.2 identification

When the substance examined is an enantiomer, the specific optical rotation is used for the identification.

If the specific optical rotation is used for identification only, the value may not be calculated on the dried substance or on the solvent free substance. The limits prescribed should take into account variation in content and purity of samples of different origin that comply with the monograph.

If the specific optical rotation test is also used to control the purity of the enantiomers, the text of the identification may mention: It complies with the test for specific optical rotation.

3.3.1.3 tests

Specific optical rotation may be used to verify the optical purity of an enantiomer. This method is less sensitive than chiral LC. In the case where one enantiomer is to be limited by the measurement of specific optical rotation, then it is to be demonstrated that under the conditions of the test, the enantiomer has sufficient optical activity to be detected. The result is calculated on the dried substance or on the solvent-free substance. Whenever possible the influence of potential impurities should be reported. Limits for the specific optical rotation should be chosen with regard to the permitted amount of impurities. In the absence of information on the rotation of related substances and when insufficient amounts of the related substances are available, the limits are usually arbitrarily fixed at ± 5 % around the mean value obtained for samples which comply with the monograph. Samples of different origin should be examined whenever possible. It is also worthwhile to examine samples which are close to the expiry date to obtain information on the influence of normal ageing.

Measurement of the angle of rotation may be used to verify the racemic character of a substance. In that case limits of +0.10° to -0.10° are usually prescribed.

If possible, it is to be demonstrated that, under the conditions of the test, the enantiomer has sufficient optical activity, to be detected.

Exceptionally the angle of rotation is used to verify the optical purity of an enantiomer, e.g. methyldopa where AlCl3 is added to increase the rotation by complex formation.

3.3.1.4 assay

Exceptionally the angle of rotation is used to assay a substance, e.g. ethambutol hydrochloride. This involves the use of a reference substance with known optical purity.

3.3.2 Ultraviolet Spectrophotometry (2.2.25)

In all cases, the suitability of the operating conditions eg. solvents employed and their quality, pH of the solution etc., must be demonstrated.

In normal use, ultraviolet spectrophotometry is a method of limited discrimination power. The use of first and second derivative techniques may increase discrimination power.

3.3.2.1 identification

Ultraviolet spectrophotometry is rarely be employed alone for identification. When it is included in an identification series, discrimination power must be demonstrated by comparing the spectrum of the analyte with spectra of similar substances. Discrimination power can be increased by using absorbance ratios rather than absorbance values.

3.3.2.2 limit test

When ultraviolet spectrophotometry is used for a limit test, it is to be demonstrated that at the appropriate wavelength the related substance to be limited makes a sufficient contribution to the measured absorbance. The absorbance corresponding to the limiting concentration of the related substance must be established.

3.3.2.3 assay

When ultraviolet spectrophotometry is used for the assay, the contribution to the absorbance of the known impurities must be evaluated. The use of specific absorbance values for assays is discouraged. If specific absorbance values are prescribed they must be evaluated by inter-laboratory trial using a batch of known purity. The purity is to be estimated by applying a variety of techniques including separation techniques and absolute methods.

3.3.3 Non-Instrumental Limit Tests

3.3.3.1 appearance of solution (2.2.1. and 2.2.2.)

These simple visual tests compare the colour (or opalescence) of the test solution to a series of standards. Normally, the test solution should be clear and colourless. These tests are intended to give an assessment of the general criterion of purity of the substance. When degrees of colour (or opalescence) are permitted, the impurity(ies) and the level to which the degree of coloration (or opalescence) corresponds is often unknown. Validation is based on the examination of batch data supplied by the manufacturer(s). However, when the impurity causing the opalescence or colour is known it may be possible to validate the visual test by comparison to a more sophisticated analytical technique.

3.3.3.2 acidity / alkalinity

This method is a test giving a general criterion of purity. It is a non-specific test used for the control of proteolytic impurities. The appropriate use of this test is adequately described above.

3.3.3.3 limit tests for anions/cations (2.4...)

These are simple and rapid tests but which are to be shown to be appropriate by recovery experiments and/or comparison with other more sophisticated methods.

Sulphated Ash (2.4.14). The sulphated ash test is intended as a global determination of cations present in organic substances but is obviously not applicable to inorganic salts of acidic organic substances. The limit is normally 0.1 per cent. This gravimetric test controls the content of foreign cations to a level appropriate to indicate the quality of production. This method can be considered to be well-established and no further validation is required.

Heavy Metals (2.4.8). Appropriate low limits must be set for the toxic elements, many of which are controlled by the heavy metal test (eg lead, copper, silver, mercury, cobalt, cadmium and palladium).

This test is based on the precipitation of these heavy metals as the sulphides and visual comparison with a standard prepared from a lead solution.

Five different procedures are described (2.4.8) and a description of these tests is given in section 2 of this Guide. Normally the limits are set at 10 ppm or 20 ppm. Lower limits may be set in which case Limit Test E is to be used. Nevertheless, it is important that the appropriate procedure is chosen for the substance to be examined and that the response is verified at the proposed limit.

The proposed test for heavy metals is performed with the sample and the sample "spiked" with lead at the desired limit. The brown opalescence produced by the sample must be less, and that produced by the "spiked" sample must be equal or more, than the standard.

It must be noted that for some of the procedures, which require incineration, there is the risk of the loss of some heavy metals eg mercury, lead in the presence of chloride. If this is likely to be the case then such metals are to be controlled by atomic absorption spectrophotometry or another appropriate instrumental technique.

When it is known that a catalyst is employed in the synthesis eg. palladium, nickel or rhodium it may be more appropriate to limit its content by a special calorimetric or instrumental method (eg. atomic absorption spectrophotometry, ICP fluorimetry etc).

Colour or Precipitation Reactions. Limit tests are also described for individual cations and anions which are based on visual comparison of a colour or opalescence. It is essential that it is demonstrated that

- the colour or opalescence is visible at the target concentration (limit)
- that the recovery of added ion is the same for the test and reference solutions (by visual observation and if possible by absorbance measurement)
- that the response is sufficiently discriminating around the target value (50 per cent, 100 per cent and 150 per cent of the target value) by measuring the absorbances at an appropriate wavelength in the visible region
- a recovery experiment at the target value is carried out six times and the repeatability standard deviation calculated. Recovery should be greater than 80 per cent and the repeatability RSD should be less than ±20 per cent.

It would be desirable when appropriate to compare the results obtained from a recovery experiment using the proposed limit test procedure with a quantitative determination using a different method eg atomic absorption spectrophotometry for cations or ion chromatography for anions. The results obtained by the two methods are to be similar.

3.3.4 Atomic Absorption Spectrometry (2.2.23)

Atomic spectroscopy is exclusively employed in tests to determine the content of specific elements which are present in substances as impurities. The following validation requirements are pertinent to atomic spectrometric methods.

3.3.4.1 specificity

In principle, this technique is specific, using the appropriate source and wavelength, for the element to be determined since the atom emits or absorbs radiation at discrete spectral lines. However, interferences may be encountered due to optical and/or chemical effects. Thus it is important to identify the interferences and, if possible, reduce their effect by using appropriate means before starting the validation programme.

Such interferences may result in a systematic error if a direct calibration procedure is employed or reduce the sensitivity of the method. The most important sources of error in atomic spectrometry are associated with errors due to the calibration process and to matrix interference. (Care must be taken to avoid memory effects).

3.3.4.2 calibration

Aqueous standards are prepared and analysed at different concentration levels, spread over the calibration range.

The number of concentration levels at which standards must be prepared depends on the calibration model used. To demonstrate the applicability of a straight-line regression model, standards should be prepared at minimum of 4 concentration levels. A parabolic regression model also requires at least 4 concentration levels. Preferably, the concentration levels are evenly distributed over the calibration range.

Generally, it is recommended to perform at least 5 measurements at each concentration level.

Calibration problems can often be detected visually. However, these plots alone cannot be used as a proof for the suitability of the calibration procedure.

a) The measured absorbances are plotted as a function of the concentration, together with the curve that describes the calibration function and its confidence interval. This curve should fit the data points.

b) The residuals, i.e. the difference between the measured and the estimated absorbance are plotted as a function of the concentration. When a suitable calibration procedure is applied, the residuals are randomly distributed around the x-axis.

When the variance on the signal increases with the concentration, as is often the case with atomic spectrometry and shown from either a plot of the residuals or with a one-tailed t-test, the most precise estimations are made with a weighted calibration model. Both linear and quadratic weighting functions are applied to the data to find the most appropriate weighting function to be employed.

For a weighted model, the weighted residuals, i.e. the weight multiplied by the residual, are plotted as a function of the concentration.

a) the measured absorbances are plotted as a weighted function of the concentration, together with the curve that describes the calibration function and its confidence interval.

b) the weighted residuals are plotted as a function of the concentration.

It must be demonstrated that the data accurately fit the model. Application of a straight line regression model implies that the linearity of the calibration line is investigated.

3.3.4.3 matrix effects

When aqueous reference solutions are measured to estimate the calibration function, it must be ensured that sensitivities in the sample solution and in the aqueous solution are similar. When a straight line calibration model is applied, differences in sensitivity can be detected by comparing the slopes of a standard addition and an aqueous calibration line. The precision of the estimation of the slopes of both regression lines depends on the number and distribution of the measurement points. Therefore it is recommended to include sufficient measurement points (certainly > 5) in both regression lines, and to concentrate these points mainly on the extremes of the calibration range.

The slopes of the standard addition line and the aqueous calibration line are compared, by applying a t-test, to check whether slopes of both regression lines are significantly different. If that is the case, then Method II-Standard Additions is to be applied and if it is not the case, Method I-Direct Calibration can be employed.

3.3.4.4 detection and quantification limit (based on the standard deviation of the blank)

To estimate the detection and quantitation limit, representative blanks are prepared and analysed. Preferably matrix blanks are used, which contain every component of the sample except the analyte. However, when no matrix blanks are available, reagent blanks, containing all reagents and prepared in the same manner as the sample solution, can be used.

Other aspects of the validation programme are covered above.

3.3.5 Separation Techniques

Chromatographic methods

The different chromatographic procedures, thin-layer, gas and liquid chromatography may be employed in the IDENTIFICATION section, in the TESTS section for the limitation of related substances and in the ASSAY section to determine the content of the active ingredient. The methods are to be validated according to the principles already described but there are particularities of the different chromatographic techniques which are to be taken into consideration.

3.3.5.1 Thin-Layer Chromatography (2.2.27)

This chromatographic technique is widely employed in the Pharmacopoeia for identification using a reference substance and for the limitation of impurities with or without the use of a reference substance. When impurities are to be determined quantitatively, appropriate

instrumentation must be employed. For the most part, silica is employed as the stationary phase but reverse-phase stationary phases eg. silanised silica gel, or cellulose stationary phases are also employed. Nonetheless, the following points are common to the application of thin-layer chromatographic techniques whether used for identification or for a test for related substances.

- Specificity - It is accepted that for an identification test, specificity cannot be attained using this technique alone but good discrimination can be expected - it must be accompanied by other tests which together assure specificity. Specificity may not be attainable for a limit test in which case (an)other test(s) must be described to control the impurity(ies) not separated. Discrimination power is to be demonstrated. For an identification test improvement in discrimination power can sometimes be achieved using a spray reagent which differentiates similar substances by colour.
- Stationary Phase - It is to be demonstrated that the test is applicable using plates of the same type but of different origin. Separations which can only be achieved on one particular type of plate are to be avoided, if possible.
- Performance Test (system suitability test) - such a test is generally performed to verify the separation of two closely eluting substances, the substance itself and a similar substance (critical pair). It is to be demonstrated that the separation of the chosen substances will guarantee the suitability of the chromatographic system. This performance criterion is essential for a test for related substances.

Additional aspects which require further documentation when this type of technique is applied to a test for related substances, include:

- Detection - The use of specific spray reagents must be avoided when applying a related substances test unless the test is designed to limit a named impurity using a reference substance.
- Detection Limit - When applying a quantitative instrumental procedure one of the described methods for the calculation of the detection limit apply. When a visual method is applied, it is to be demonstrated that the quantity corresponding to the specified limit is detectable.
- Response Factors - if the known impurities are available then the similarity of response factors (relative to the substance itself) is demonstrated using the given detection conditions. For a limit test, differences in response can be shown by comparison of the visual detection limits.
- Quantification Limit, Linearity, Range and Repeatability - data are also required when an instrumental quantitative thin-layer procedure is applied.

3.3.5.2 Liquid Chromatography (2.2.29)

This chromatographic technique is usually applied to limit the content of impurities in a substance (employing an external standard, usually an appropriate dilution of the test solution), to determine the content of a substance (employing an external standard), and occasionally as an identification by cross-reference to one of the aforementioned procedures. Attention is to be paid to a number of aspects peculiar to liquid chromatography.

3.3.5.2.1 identification

- Specificity - It is accepted that for an identification test, specificity may not be attained using this technique but good discrimination can be expected. It must be accompanied by other tests which together ensure specificity. Discrimination power must be demonstrated with retention times, relative retentions or mass distribution ratio of similar substances, and the substance itself, being reported. Such information is to be supplied for a variety of stationary phases of a similar type.

3.3.5.2.2 limit Test

- Specificity
    - Discrimination power of the separation. Separation of known and potential impurities from the substance itself and if possible, from each other must be demonstrated. Specificity may be assured by detection by mass spectrometry. (An) Impurity(ies) not separated from the substance must be controlled by another method. The retention times, relative retention times or mass distribution ratio of the substance and the impurities must be reported. Such information is to be supplied for a variety of stationary phases of a similar type.
    - Discrimination power of the detection system. The choice of the detector or the detector conditions employed must be justified (eg. change in the detection wavelength when using UV detection) whilst specificity can be assured by the use of detection by mass spectrometry.
- Response Factors — it is essential to demonstrate the similarity of response of the substance and known impurities (at the wavelength of detection for UV detection but applies also to other detection systems eg refractive index, conductimetry). A response factor of a known impurity which is greater than 1.2 or less than 0.8 than that of the test substance, may require the use of either correction factors or the use of that individual impurity as an external standard when the proposed limit is 0.1 per cent or greater.
- Detection and Quantification Limits — these limits must be determined for the external standard which is either a dilution of the substance to be examined or a known impurity. When a peak of an impurity elutes close to the peak of the substance, particularly if it elutes after the peak due to the substance, detection and quantification limits are to be determined on this impurity. One of the methods for calculation of both the detection limit and the quantification limit are applied.
- Stability — data should be provided demonstrating the period of use of reference and test solutions.
- Recovery — when an extraction procedure is employed, a recovery experiment using known and available impurities is to be carried out under optimal conditions and the results reported. It is to be demonstrated that the recovery is consistent with an acceptable precision.

- Derivatisation — When pre- or post-column derivatisation is employed, it is important to establish the optimal reaction conditions (time and temperature) and also investigate the stability of the derivative under normal conditions of use.
- System Suitability Test — as described for thin-layer chromatography. The use of the signal to noise (S/N) ratio is only required when the detection limit and the specified limit are similar.

3.3.5.2.3 assay

- Specificity — this is preferable but not essential provided that the interfering impurity is present at a low level and is controlled by another test.
- System Suitability Test — as described for thin-layer chromatography.

Limit test and assay must be validated as described above for linearity, repeatability and reproducibility.

3.3.5.3 Gas Chromatography (2.2.28)

3.3.5.3.1 identification

Specificity — the same applies here as is already described for liquid chromatography.

3.3.5.3.2 limit tests

- Specificity — see liquid chromatography.
- Response Factors (see liquid chromatography) — response factors relative to the substance itself must be provided. This is particularly important when using selective detectors eg ECD, NPD etc,.
- Detection and Quantification Limits — the same applies here as is already described for liquid chromatography.
- Stability — see liquid chromatography.
- Derivatisation — as described under liquid chromatography.
- Internal Standard — it is to be demonstrated that under the chromatographic conditions employed, the peak due to the internal standard does not interfere with the impurity peaks or that due to the substance itself.
- Recovery parameters - as described under liquid chromatography.

3.3.5.3.3 System Suitability Test

Details which are to be provided of chromatographic criteria to which a user must conform to successfully apply the test.

- Signal to Noise Ratio (S/N) is usually determined for a signal which is equal to or greater than the detection limit.
- Resolution between the peak due to the substance and a closely eluting peak of an impurity or the peak due to the substance and the peak due to the internal standard. It is also useful to give the acceptable range of values for the symmetry factor when it is

different from the accepted range of 0.8 to 1.2 as given in the general text (2.2.29). This is particularly important when employing packed columns and when the peak of an impurity to be controlled elutes immediately after the principal peak. Verification of performance using a similar column, when possible, is recommended.

- Head-Space Injection Technique - this type of injection is employed for highly volatile substances. It is important to demonstrate that the temperature and time of pre-heating of the injection vial results in equilibrium conditions. It should also be demonstrated if there is (or not) a "matrix" effect. A means of validating the head-space injection conditions is by carrying out multiple head space extractions (after each injection the head space is vented and the vial is re-equilibrated before re-injection of the gaseous phase). The pre-requisite for good conditions is that the relationship of the logarithms of the areas of the analyte peak to the number of extractions, is linear with a co-efficient of regression of 1.0. Matrix effects can be overcome by the use of the standard addition technique.

3.3.5.3.4 Assay

- Specificity - as for liquid chromatography.
- Performance Test (System Suitability Test) - as described for thin-layer chromatography.

Limit test and assay should be validated as described in Validation Section B for linearity, repeatability, reproducibility.

TEST PROCEDURES FOR THE IDENTIFICATION AND CONTROL OF RESIDUAL SOLVENTS (2.4.24).

The sample preparation and gas chromatographic systems employed are to be validated for the substance under study by applying the criteria given above (5.1.3) with particular respect to:

- specificity
- detection and quantification limits
- recovery
- repeatability
- linearity when employed quantitatively.

3.3.6 Semi-micro determination of water (2.5.12)

A number of commercial Karl Fischer reagents are available so it is important to ensure their suitability for use by means of a validation procedure such as standard addition:

Standard addition

Determine the water content (m_{H_2O}) of the sample using the proposed conditions. Then under airtight conditions add a suitable volume of a standardised solution of water in methanol R and determine the water content m_i as mg water. Repeat this step at least five times.

Calculate the regression line of the cumulative water determined against the water added. Calculate slope *b*, intercept *a* with the ordinate and the intersection d of the extrapolated calibration line with the abscissa.

The slope *b* is to be between 0.975 and 1.025 (deviation ± 2.5 %) to be acceptable. The percentage errors e_1 and e_2 are less than ± 2.5 per cent.

$$e_1 = \frac{a - m_{H_2O}}{m_{H_2O}} \times 100$$

$$e_2 = \frac{d - m_{H_2O}}{m_{H_2O}} \times 100$$

Calculate the recovery of each standard addition step. The mean recovery is to be within 97.5 per cent and 102.5 per cent to be acceptable.

3.3.7 Volumetric titrations (2.5.11; 2.2.19; 2.2.20)

When developing a new volumetric assay method, it is recommended to titrate at least seven different quantities under the prescribed conditions in a randomised order to give end point volumes in the range of 20 per cent to 90 per cent of the volume of the burette employed. Subsequently, the data are treated statistically and a number of criteria are to be fulfilled to permit acceptance of the titration procedure.

The relative error in reading of the weight on the balance and of the volume at the end-point is to be less than 0.5 per cent of the values found.

The results, as end-point volumes 'V_i' in dependance of weight 'm_i', are evaluated by linear regression. The regression line is calculated and characterised by the slope 'b_{obs}', the extrapolated intercept 'a_{obs}' and the precision as sdv(v).

1st Criterion – Proportional Systematic Error (Bias)

The calculated slope 'b_{obs}', taking into account the titre of the standardised volumetric solution, is within 0.3 per cent for potentiometric tritrations (0.5 per cent for visual titrations) compared to the theoretical value given as titration constant 'b_{theor}'.

$$\left(\frac{b_{\text{obs}} - b_{\text{theor}}}{b_{\text{theor}}}\right) \times 100$$

where $b_{\text{theor}} = \dfrac{Z}{M_r C_r}$

M_r is the relative molecular mass, Z is the stoichiometric factor of the chemical reaction and C_r is the molar concentration of the titrant.

2nd Criterion – Additional Systematic Error (Bias)

The extrapolated intercept a_{obs} is less than 0.4 per cent for potentiometric titrations and 0.6 per cent for visual titrations of the expected or target titration volume. This criterion may not be fulfilled when the titration is carried out too rapidly (potentiometric titration) or an unsuitable indicator has been employed (visual titration).

$$\left(\frac{a_{\text{obs}}}{V_T}\right) \times 100$$

a_{obs} is the extrapolated intercept of the regression line at zero and V_T is the expected or target titration volume.

3rd Criterion – Precision (Statistical Error)

The remaining estimated standard deviation sdv(V) is less than 0.3 per cent for potentiometric titrations (0.5 per cent for visual indicator titrations) of the mean titration volume of end-point using the titration procedure to be introduced in the monograph.

$$\left(\frac{sdv(V)}{V_T}\right)\times 100$$

sdv(V) is the estimated standard deviation.

$$\mathrm{Sdv}(V) = \sqrt{\frac{Sdd}{n-2}}$$

$$\mathrm{Sdd} = \sum (V_i - a_{obs} - b_{obs} m_i)^2$$

V_i is the titration volume, m_i is the mass of the substance and n is the number of titrations performed.

4th Criterion – Pratical Relative Error

Some titration procedures may not fulfil the first and second criteria but exhibit low and acceptable bias at the target titration volume (8 ml ± 1 ml for a 10 ml burette). Thus, the first and/or the second criteria given above are not met, then calculate the relative accuracy at the target titration volume.

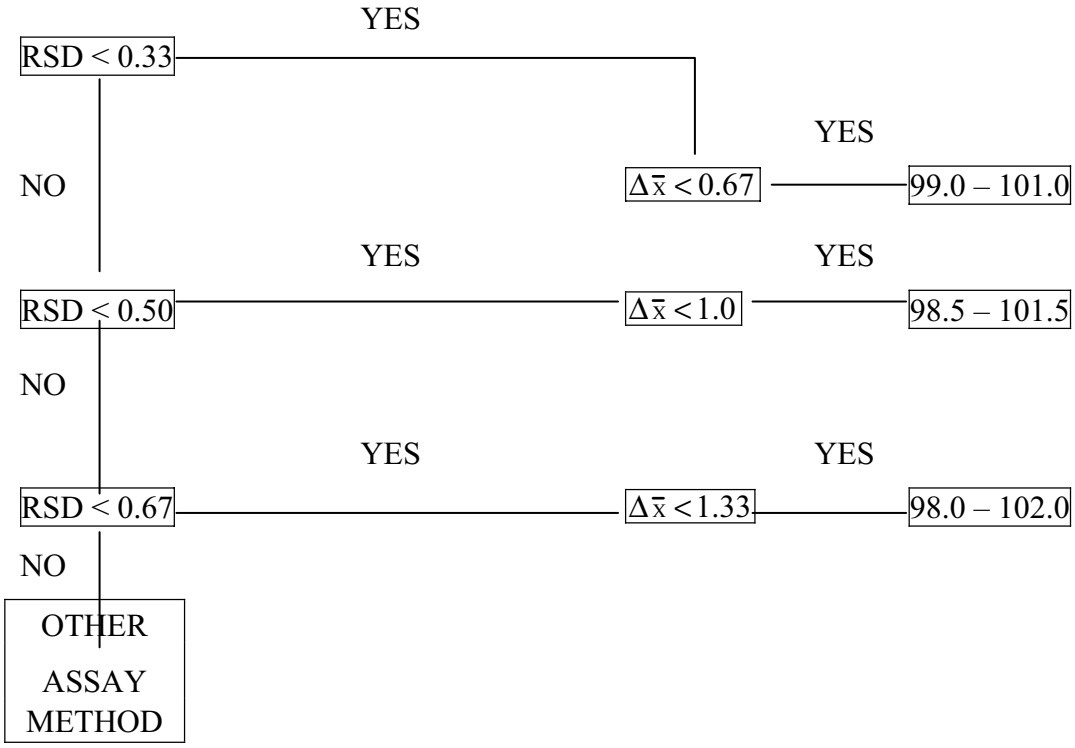
$$\left|\left(\frac{a_{\mathrm{obs}}}{V_{\mathrm{T}}} + \frac{b_{\mathrm{obs}} - b_{\mathrm{theor}}}{b_{\mathrm{theor}}}\right)\right| \times 100$$

However, when the volumetric titration procedure is well established it is sufficient to verify that the repeatability and accuracy of the titration (a minimum of six replicates) are not greater than the limits given in the table below and also given in the decision tree.

VOLUMETRIC TRITRATION	CONTENT LIMITS (PER CENT)	REPEATABILITY (RSD)	RELATIVE ACCURACY (PER CENT)
ACID/BASE	± 1.0	0.33	± 0.67
NON-AQUEOUS	± 1.0	0.33	± 0.67
CONJUGATE ACID OF BASE	± 1.0	0.33	± 0.67
REDOX	± 1.5	0.5	± 1.0
ARGENTOMETRIC	± 1.5	0.5	± 1.0
COMPLEXOMETRIC	± 2.0	0.67	± 1.33

The figures in the table are given as guidance and it may be demonstrated that stricter limits can be applied. The use of volumetric titrations is applicable only when it has been demonstrated that impurities are present at low levels, otherwise other assay methods are to be introduced.

DECISION TREE FOR VALIDATION OF VOLUMETRIC TITRATIONS

Repeatability RSD (n = 6)

Relative accuracy $\Delta\bar{x} = \frac{\bar{x} - x_{theory}}{x_{theory}}$